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LETTERS TO THE EDITORS

THE PROGRAM OF THE CERVICAL CARCINOMA *IN SITU* IN PREGNANT WOMEN

TO THE EDITORS:

During the period from 1952 to 1958 in the Oncologic Out-Patient Center at Gliwice, Poland, 23,504 women with erosions were examined. There were 309 cases of preinvasive carcinoma detected and 114 so-called border-line cases (casus limitans, atypia majoris gradus suspecta quoad carcinoma in situ).

Among the cases of cancer and suspected cancer were 37 pregnant women. The average age of the patients was 29. All the patients were multiparas. Of the patients under observation, 26 went to term, 8 cases had an interrupted pregnancy and 3 are still pregnant or post partum. Preliminary cytological and histopathological examinations were made and after exclusion of invasive cancer the patients were called in for follow-up examinations every four weeks. The histopathologic examination, which was decisive for the further management of the individual case, was made 12 weeks post partum. Out of 37 patients the first biopsy revealed preinvasive carcinoma in 24, and in 13, casus limitans or atypia suspecta quoad carcinoma in situ. Systematic examinations were completed in 30 women, four women did not come for the check-up examination and four others are still under observation. In 30 postpartum patients the changes in the epithelium, labeled as carcinoma in situ and casus limitans, did not disappear. Of the 24 women with diagnosed preinvasive cancer during pregnancy, carcinoma persisted in 17 cases, in three other cases casus limitans or atypia majoris gradus was found, and in four women the examinations are not completed or the patients did not return for the follow-up examination. Of the 13 women in whom atypia suspicious for cancer was diagnosed during the preliminary examination, cancer was found in four post partum, in six, atypia of considerable degree persisted, and the other three patients have not completed the examination or did not return for the check-up. In three cases in which carcinoma in situ was diagnosed during pregnancy, only atypia majoris gradus was found post partum. This does not prove that the pathologic lesion regressed, because it also happens that in non-pregnant women the preliminary examination of four-quadrant specimens reveals carcinoma in situ, and in the postoperative material cancer is not found. In the Institute of Oncology at Gliwice, Poland, out of 120 cases of cancer in non-pregnant women, the postoperative specimen after conization did not reveal cancer in 14 cases (12%); only atypia majoris gradus was diagnosed. In 13 pregnant women the preliminary examination revealed atypia and after delivery early cancer was diagnosed in four cases. This does not mean that there must be progress in the neoplastic disease in pregnancy since this also occurs in non-pregnant women. In the Institute at Gliwice out of 130 cases of non-pregnant women, preliminarily diagnosed as having atypia, preinvasive cancer was found in the postoperative material after conization, in 46 cases (35%). Out of 37 pregnant women under observation the examination was completed in 30 and the atypical epithelium found during pregnancy did not become normal post partum. On the basis of our previous observations we are of the opinion that the same criteria in the diagnosis of cervical carcinoma in situ are binding in pregnant as well as in non-pregnant women, and pregnancy itself does not bring about changes which would make diagnosis difficult. We did not notice in the women under observation any distinct progress of preinvasive cancer during pregnancy. Therefore we allow full-term pregnancy in cases with carcinoma in situ whenever the systematic follow-up examination is possible. A biopsy six weeks post partum for the purpose of excluding invasion must be performed. The final pathologic examination to determine treatment must be done 12 weeks after delivery.

Thus, reports of Epperson and associates (1) and Nesbitt and associates (2) have not been confirmed in our experience. On the other hand, other authors (3, 4, 5, 6, 7, 8, 9, 10) report results of their investigations similar to ours and believe, as we believe, that carcinoma in situ in pregnancy is not a reversible lesion.

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Maria Kawecka, M.D.
Instytut Onkologii
ul. Czerwonej Armii 15
Gliwice, Poland

CYTOLOGY OF TUBERCULOUS ENDOMETRITIS

TO THE EDITORS:

Having read the paper on "Cytology of Endometritis Tuberculosa" (Vol. II, No. 3, pp. 526-529) we went to our files and re-examined the vaginal and cervical smears of ten (untreated) patients with this condition. In none of these cases had the diagnosis been made by exfoliative cytology. Therefore, we agree with Iklé (p. 528) that cytology offers little in detecting endometrial tuberculosis. Nevertheless, we want to stress several points:

I. Intense desquamation of endometrial cells (Terzano, p. 526) was never obvious in our material.

II. The presence of large numbers of polynuclear leukocytes, lymphocytes, plasma cells, small histiocytes and red blood cells as well as blood pigment and cellular debris was noted in six cases. These changes are not at all characteristic for tuberculosis as they are readily seen in non-specific inflammation. Moreover, trichomoniasis (which was present in seven patients) may (at least in part) be held responsible for these findings.

III. Multinucleated giant cells (Terzano p. 526; Iklé p. 528) were found in two cases.

We fully agree with Campos (p. 528) that these cell-types are occasionally seen in a variety of non-specific inflammations of the female genital tract.

Our material includes smears from a 64-year old patient with tuberculosis of the ectocervix and the endometrium. These specimens remind us in many respects of the cases illustrated by Iklé (p. 528). Incidently, the cervical lesion was originally mistaken for cancer. The cervical scrapings (Class II) were note-worthy as they contained large numbers of very active looking polymorphic small histiocytes, multinucleated giant cells and epitheloid cells. In such cases a tentative diagnosis of tuberculosis would have been made.

Michel Thiery, M.D.
Department of Obstetrics and Gynecology
The State University of Ghent,
Ghent, Belgium

DEFINITION OF A "KERATINIZED CELL"

TO THE EDITORS:

Reference is made to the Terminology Issue (Vol.II, No. 1, 1958) of ACTA CYTOLOGICA.

1. We have the feeling that the Members of the Terminology Sub-Committee are not always referring to the same cellular type. According to Berger (p. 57) the keratinized cell has a visible nucleus; for Graham (p. 57) it is always anucleated. "True keratinized cells" (skin; estrous vaginal epithelium of the mouse; leukoplakia and prolapse), to use Papanicolaou's terminology, (Vol. II, No. 3, p. 464-465) stain orange, yellow or ochre and are anucleated, whereas "cornified cells" stained pink and still have a (sometimes fading) nucleus. Morphologically both cellular types are not identical. Therefore the terms "anucleated (squamous) cell" or "squame" should be used only for designating the "true keratinized cell."

2. Intercellular bridges do not exist in squames, as clearly demonstrated by electron microscope studies of ultra-thinly sectioned exfoliated material. The dense cellular membrane of anucleated squamous cells is undulated (microvilli) (Fig. 1). At the apex of several microvilli thickenings of the cell

membrane are to be seen (Fig. 2) which are remnants of the classical attachment plaques found in deeper cell layers.

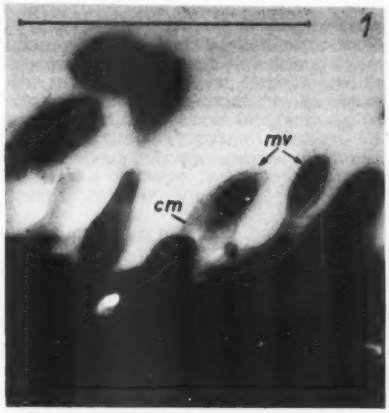


Fig. 1. Ultra-thin section of squames exfoliated from the vaginal epithelium of the estrous mouse (m - microvilli; cm-cell membrane) (x 60.000).

Fig. 2. Portions of two exfoliated squames of same origin (m - microvilli; is - intercellular spaces; t - thickenings of cell membrane; cm - cell membrane) (x 40.000).

A. Lagasse, Sc. D.
Laboratory of Electron Microscopy
State University of Ghent,
Ghent, Belgium

Michel Thiery, M. D.
Department of Obstetrics & Gynecology
The State University of Ghent,
Ghent, Belium

REMARKS ON THE NEW CYTOLOGICAL NOMENCLATURE WITH SPECIAL CONSIDERATION GIVEN TO THE DETERMINATION OF THE KARYOPYKNOTIC INDEX

TO THE EDITORS:

According to the suggestions which appeared in ACTA CYTOLOGICA, Vol. II, No. 1, superficial cells are considered to be only those which display pyknotic nuclei, are polygonal in shape and stain either cyanophilic or eosinophilic. The Karyopyknotic Index thus is identical with the Superficial Cell Index. The Eosinophilic Index is derived from the percentage of eosinophilic cells among the superficial cells. For instance, with a Karyopyknotic Index of 40% and the amount of eosinophilic cells at 80%, the Eosinophilic Index will be

$$\frac{40 \times 80}{100} = 32\%$$

Thereby the Karyopyknotic Index becomes a very important criterion for the definition of various cell forms and the evaluation of the hormonal situation. The various methods of determining the Karyopyknotic Index and their influence on the widely varying reports on the index have been referred to in ACTA CYTOLOGICA. However, the problem did not seem to be outlined in its full significance.

We have determined the Karyopyknotic Index on 60 slides according to four different methods:

1. By estimation, i.e., by subjective evaluation of the shrinkage and condensation of the nucleus - towards a structureless mass - using a 40X objective and 8X ocular (Murray and Herrenberger, Roth).

2. By measuring all nuclei up to a diameter of 5 mμ under 100X oil immersion and 6X ocular (Ferin).

3. By measuring all nuclei up to a diameter of 6 mμ under 100X oil immersion and 8X ocular (Pundel).

4. By means of phase contrast microscopy by counting the bright red-appearing nuclei in Papanicolaou slides under 40X objective and 8X ocular (Wied).

The following average values have been obtained:

By estimation: 50.5%
By counting nuclei up to 5 mμ: 50.02%
By counting nuclei up to 6 mμ: 62.12%
By means of phase contrast microscopy: 31%

There was no constant relationship between the indices as determined by the various methods. However, the values obtained by phase contrast microscopy were significantly the lowest in all preparations. We considered only those nuclei pyknotic which gleamed brightly red, without leaving a dark shadow in the background. The estimated values corresponded approximately with the ones up to 5 mμ, whereas the 6 mμ values were slightly higher.

The nuclear sizes as determined by means of phase contrast microscopy showed the following distribution:

3mμ - 4.4%
4mμ - 64.3%
5mμ - 30.0%
6mμ - 1.3%

The range of size in the individual slides has not been greater than ±5%. with the other methods nuclei of 5 mμ diameter were in the majority.

Pundel and Lichtfus have been performing similar comparative studies. They found agreement between the values obtained by means of phase contrast microscopy and the ones obtained by counting nuclei up to 6 mμ, whereas the 5 mμ values lie considerably under this magnitude.

The striking differences between the results of Pundel and Lichtfus and our own can be traced, as preliminary investigations show, to the application of different staining methods. We used a modified Papanicolaou technique which yields very transparent cell pictures and extremely find nuclear staining. Pundel and Lichtfus, on the other hand, used the Shorr hematoxylin staining technique.

To attack this problem slides from the same patients were stained according to the different methods and the indices again determined in the four ways described above. As staining techniques we used:

1. The routine Papanicolaou technique,
2. The same technique, only with a longer staining time in hematoxylin,
3. The Shorr hematoxylin technique.

The following table shows the results of these studies:

Staining technique	Phase contrast microscopy	Estimated	Up to 5 mμ	Up to 6 mμ
1. Papanicolaou with 3 min. hematoxylin	16%	31%	28%	56%
2. Papanicolaou with 8 min. hematoxylin	30%	34%	27%	58%
3. Shorr hematoxylin	49%	45%	30%	57%

TABLE 1.

From these results it becomes clear that no matter what staining procedure is used the Karyopyknotic Index varies considerably with the method of determination. As expected, the values obtained by counting the nuclei up to 5 mμ, and 6 mμ respectively, are basically the same with all staining methods. The differences noted are within the normal range of values. Also the values estimated with normal microscopy show only a slight elevation by application of more intensive nuclear staining.

The determination on the Karyopyknotic Index by means of phase contrast microscopy reveals higher values even by prolongations of the hematoxylin staining time. By staining according to Shorr the Index is much higher, three times in the quoted example and thereby almost reaches the 6 mμ values. Thus we are able to fully reconfirm the results of Pundel and Lichtfus, which at first glance appear surprising. With the Papanicolaou technique, all values when determined by means of phase contrast microscopy are considerably lower than with the other methods mentioned. In the most extreme example the Karyopyknotic Index was 16% when determined by phase contrast microscopy as compared to 75% for the 6 mμ value. The smallest difference between both procedures was between 52 and 67%.

We think it is very important to discuss these questions again on an international basis and to determine exactly which method to use for determination of the Karyopyknotic Index. Without this, all the efforts for standardization of the nomenclature in regard to the definition of superficial cells will remain in vain. One would only replace one factor of uncertainty, i.e., the difficult and mainly subjective differentiation between intermediate cells and "superficial cells with vesicular nuclei" by another factor: the dependance of the Karyopyknotic Index upon the staining method applied.

Which methods can be used for the objective determination of the Karyopyknotic Index and what are their advantages or disadvantages?

1. The estimation of the Karyopyknotic Index with the standard microscope offers fairly good and comparable results to the individual investigator. The determination of the index is quickly done and can be performed even though no phase contrast microscope is available. However, because of the fluctuations caused by the differing evaluation from one investigator to the other, this method will have only little use for exact cell definitions in scientific studies (Pundel).

2. By counting all nuclei up to a diameter of 5 mμ or 6 mμ we obtain an objective standard by which the reports of different authors become comparable. It is, however, required that the measurements be performed very exactly. We experienced that even by using different objectives of the same manufacturer (with the same designations) slight differences in the measurements are obtained, resulting in deviations of the Karyopyknotic Index. Thus, in any case, the ocular micrometer has to be calibrated for each given optical combination. From this it follows that exact nuclear and cellular measurements are very time consuming and therefore are out of the question for routine use, but may well be applied for scientific investigations.

Finally, it has to be borne in mind that by size measurement only a magnitude is determined, but not the nuclear pyknosis. From the length and the size of a person's shoes one can make only limited conclusions towards his degree of maturity or his age. In a similar way one may only make very cautious conclusions from the nuclear size as to the maturity of the cell. This may be a weak comparison, but it contains a grain of truth. For the determination of the Karyopyknotic Index by measurement of nuclear sizes in Papanicolaou stained slides, the count of nuclei up to 5 mμ seems to be the best method. Among the nuclei up to 6 mμ in diameter we found many which were not pyknotic. The situation seems to be different in regard to the Shorr hematoxylin and the other staining methods.

3. The determination of the Karyopyknotic Index on the stained smear under the phase contrast microscope has, in our opinion, two essential advantages. In this method the real Karyopyknotic Index and not only a quantity of nuclei is determined. Furthermore, the method is simple and fast. The disadvantage is that it yields widely varying results according to the staining method applied. If an agreement should be achieved to use this method for the determination of the Karyopyknotic Index, the staining method should also be agreed upon. If, for some reason, one author deviates from the recommended staining method, he should always note this fact.

Which one of the mentioned staining procedures eventually will be used is of minor importance. Important for the mutual understanding is simply the standardization of the staining method used.

I would like to take this opportunity to discuss another problem of cytological nomenclature. According to the new rules for cytological nomenclature the group of intermediate cells is comprised of cells of greatly varying size and shape. These start with the small polygonal cells, which are hardly larger than parabasal cells, and continue so as to include as the largest, ones which almost completely resemble superficial cells, differing from these only by their vesicular nuclei.

Disagreement arose over the question as to how to differentiate intermediate from superficial cells; the reason being that many cytologists (i.e., Boschann, Messelt and Zinser) believe that this group does not represent a uniform entity. We share this opinion. Cells of the size of superficial cells are doubtless the expression of a higher degree of maturation or a different hormonal situation than the much smaller cells from the deeper layers of the intermediate zones. Therefore we distinguish:

1. large intermediate cells: all eosinophilic and cyanophilic cells of the size of superficial cells with vesicular nuclei, which by many authors have been thought to come from the deeper superficial layer;

2. small intermediate cells: herein we include all small polygonal cells with a vesicular nucleus, i.e., those which, by many authors, have been considered intermediate cells exclusively.

We support the recommended international nomenclature for superficial cells as a basis for mutual understanding. Furthermore, in this way we obtain the possibility of liberally distinguishing between the various forms of intermediate cells. Yet, we are still aware of the fact that the distinction between large and small intermediate cells may be influenced by individual judgement.

Hans Jürgen Soost, M.D.
I. Universitäts-Frauenklinik
Munich, Maistrasse 11, Germany

CLASSIFICATIONS OF THE CYTOLOGICAL REPORTS

TO THE EDITORS:

Reference is made to the Section entitled CYTOLOGICAL REPORTS, THEIR IMPLICATIONS IN CANCER, HORMONAL AND MICROBIOLOGICAL SCREENING AS EVALUATED BY 22 CLINICIANS, in ACTA CYTOLOGICA 2:141-159, 1958.

The majority of the clinicians participating in this opinion poll stated that they prefer the five Classes of Papanicolaou for cytological reports from their laboratories, as compared with the three

SMEAR NO. _____

UNIVERSITY OF CALIFORNIA CYTOLOGY LABORATORY
CANCER RESEARCH INSTITUTE
SAN FRANCISCO HOSPITAL

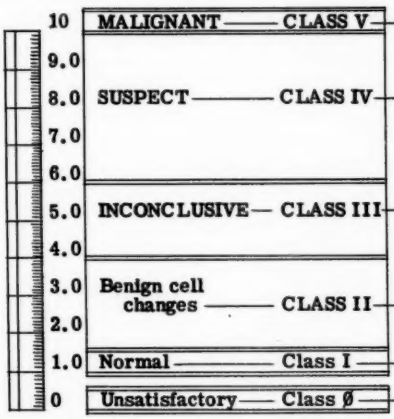
CYTOLOGIC STUDY

Patient's name _____ Hosp. No. _____ Wd. _____

Age _____ Sex: _____ Source of specimen _____

Date of collection _____ Referred by: Dr. _____

DIAGNOSIS



CLASS V: CONSISTENT WITH MALIGNANCY
*Biopsy Indicated *

CLASS IV: Abnormal cells are seen which are highly suspected of being malignant. *Repeat requested; Biopsy indicated.

CLASS III: A typical cells are present, the precise differentiation between benign and malignant changes cannot be made.
*Repeat requested.

CLASS II: Slightly a typical cells are present which are within the limit of non-malignant changes.

CLASS I: Typical cells observed.

CLASS Ø: Unsatisfactory: _____

DIAGNOSIS: CLASS _____ , _____ Points

COMMENT:

RECOMMEND:

_____ M. D.

*NOTE: The scale at left indicates the degree of observed cellular alterations and DOES NOT suggest the percent chance of the patient having cancer. A "positive" smear should not be regarded as diagnostic of cancer. It is valuable presumptive evidence of cancer and correct in a high percentage of cases. The diagnosis should be confirmed or disproven by biopsy.

classifications (negative, doubtful, positive). There was also an interesting discussion on the other questions in this opinion poll.

In my opinion, much of the problem remained still unsolved because we have yet to establish a uniformity among the cytologists as to exactly what they mean with the various classifications. Is, for example, Class III "inconclusive" (i.e., "I don't know") or is it "suspicious" (i.e., "I suspect malignancy, but I am not sure")? Another question comes to mind concerning Class II: does a smear which is classified Class II convey any, even the slightest, warning to the clinician, or is it just a slightly abnormal normal, but definitely normal specimen? Similarly, one might ask a question concerning Class IV: does a specimen classified Class IV definitely indicate the presence of malignancy, only that the cells are less abundant than in a specimen of Class V, or is Class IV qualitatively less "malignant" than Class V?

The American Registry of Medical Technologists (of the American Society of Clinical Pathologists) gives tests to cytotechnologists, in which - I was told - the Class III report is what the cytotechnologist would give to lesions such as dysplasia or carcinoma in situ.

During a visit at the University of California I had the opportunity to see a report sheet which I submit herewith (reprinted with the permission of Dr. David A. Wood - Ed.) in which the cytological reports are not only given in the routine classification of Papanicolaou, but also in "points" which are evident by the calibration of the report on the left side of the form.

I feel that this report form from the University of California Cytology Laboratory emphasizes excellently the necessity for further interpretation of the Papanicolaou classifications if they are to be uniformly meaningful to all clinicians.

I would be very interested to hear if one should consider a Class III report to indicate:

1) suspicious of malignancy, but not definite, OR
2) atypical cells found which still might be benign, OR
3) atypical cells found which apparently derive from dysplasia or carcinoma in situ (but not from invasive carcinoma), OR
4) completely inconclusive (i.e., limitation of the cytological technique).

It seems especially important to settle the problem of the Class III report first, since many of the publications on diagnostic accuracy depend on a uniformity of classification. For example, is a Class III report a "false positive" finding if we find, histologically, only metaplasia or dysplasia, or is it a "correct" report? On the other hand, is a Class III report a "false negative" finding, if the histological section reveals an invasive lesion? Certainly the cytologist cannot expect to have a classification in which he is correct in any case. Where is the sharp dividing line? Above Class III, below Class III, or within Class III?

May I ask the Editors of ACTA CYTOLOGICA to treat this subject in an early discussion?

José R. del Sol, M. D.
Alberto Bosch 10
Madrid, Spain

Re: Letter from Dr. José del Sol

Since I do not use the five classes of Papanicolaou I am not sure that my remarks will be pertinent to this discussion. Our reports read as follows:

Positive, consistent with adenocarcinoma
Positive, consistent with squamous carcinoma
Positive, undifferentiated cancer cells present
Doubtful
Negative

The positive reports are self explanatory, as are the negative. I agree with del Sol that it is in Class III or doubtful categories that there needs to be clarification. In our laboratory, there are two "automatic" doubtful reports. One such cellular picture is the presence of endometrial cells in a postmenopausal woman. The second is the presence of dyskaryotic cells only, no third type differentiated squamous cancer cell, fiber or undifferentiated cells being present. If a repeat smear on a postmenopausal patient with endometrial cells shows again only benign endometrial cells we call the smear negative and make a note of the presence of the endometrial cells on the report slip. If a patient who has only dyskaryotic cells continues to have only dyskaryotic cells the smear remains doubtful. We believe that we are identifying the cells accurately but we are "in doubt" because there is no clear understanding of what this lesion represents.

Other than these two cellular pictures the doubtful or Class III report means just what it says. We are in doubt because the cells show some atypicalities but are not characteristic enough to be called malignant. All our doubtful smears are repeated as nearly as possible. It may be of interest that 14% of our doubtful smears are positive on repeat, 86% negative. Because our laboratory makes a great effort to repeat the doubtful smears and because no charge is made for repeat smears, in a series of 8000 cases only 80 or 1% were left in this category. In this group of eighty cases it was impossible to repeat the smear, usually because the patient had been operated on before a repeat smear could be obtained.

I agree with del Sol that it is difficult to give accurate statistics when a large number of cases are in Class III. If a patient with an invasive carcinoma has a Class III report, is it correct because something abnormal was seen or is it wrong because no definite cancer cells were identified? The method loses much of its specificity when large numbers are placed in the Class III category. I believe that doubtful, suspicious or Class III smears should be repeated and a definite diagnosis made in almost all cases.

Ruth M. Graham, Sc.D.
666 Elm Street
Buffalo, New York, U.S.A.

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Vol. III ORIGINAL ARTICLE No. 2

The simultaneous separation and concentration of corpuscular elements and bacteria from sputum

S. RASTGELDI, J. A. TOMENIUS AND G. WILLIAMS

Secretion from the bronchial mucosa of the healthy human subject is rarely available for cytological investigations as, normally, it is swallowed together with the secretions and exfoliated cells of the upper respiratory and alimentary tract. The volume and nature of normal bronchial secretion varies both with the external environment and the amount of stimuli from within (4). Sputum from patients suffering from various disorders of the bronchial tree is easy to obtain in sufficient quantities for cytological and chemical investigations. Bronchial cytology has attracted attention during recent years. With the exception of Muller's (11) original work (1896), no substantial work on the composition of the mucous ground substance of sputum has been done, until Brogan (2) recently investigated the carbohydrate complexes of the bronchial secretion.

Figure 1: Suspension of mucus in physiological saline before and after treatment with 5 ml 10% hydrogen peroxide at 37° C.

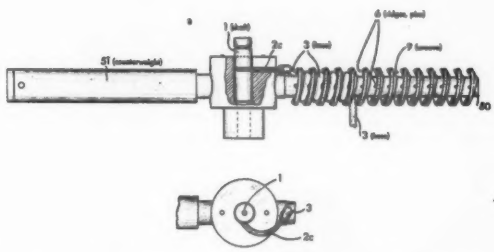

Figure 2: Model constructed to demonstrate the principle of threshold centrifugation. The tubular inlet is designated by 1. A bent tube 2c connects the tubular inlet with the plastic tubing 3 which is laid in the helically formed groove 9. The peripheral wall of the groove is provided with a large number of ridges or pins 6. These ridges press the corresponding points on the peripheral wall of the plastic tubing 3 towards the center of rotation and divide the tubing 3 into a large number of compartments which correspond to a large number of intercommunicating shallow centrifuge tubes. During centrifugation the suspension is continuously fed through the inlet tube 1. The speed of the centrifuge being kept constant, the relative centrifugal force, R.C.F., increases progressively from the central end of the tubing 3 towards the peripheral end according to the formula $R.C.F. = 1.117 \times 10^{-6} \times r \times N^2$, where r (radius) corresponds to an abutment or a threshold in the length of the tubing 3 and N to revolutions per minute. Heavier particles in a suspension running continuously through the tubing 3 are precipitated centrally and lighter particles more peripherally on the peripheral wall of the tubing. The suspending agent cleared of the particles is ejected from the peripheral end of the tubing.

From the Chemistry Department II of the Karolinska Institutet (Head: Professor Erik Jorpes) and the Medical Out Patient Department of the S:t Gorans Sjukhus, Stockholm, Sweden.

Himmelweit (7) found the mucin to contain a carbohydrate complex which antagonized the actions of the hemagglutinins of influenza virus A and B. His work has been confirmed by Curtain, Marmion and Pye (3). A similar carbohydrate complex in human urinary mucoprotein has been isolated by Tamm & Horsfall (27) and has been found by Perlman, Tamm & Horsfall (15) to be electro-phoretically homogeneous at pH 6.8 and 8.6. Both Gottschalk (5) and Odin (12) have found this inhibitory carbohydrate to be a component of the carbohydrate residue of the urinary mucoprotein. Odin has stated that the substances giving the characteristic reactions of sialic acid are concerned in the inhibition of virus hemagglutination.

In his fundamental work on the carbohydrate complexes of bronchial secretion, Brogan (2) collected a total of 1216 specimens, of which 385 selected and pooled samples yielded 28.6 g of acetone dried sputum. This dry powder was suspended in water (concentration 5 g/liter) and digested by pepsin at 37° C, pH 2.3, for 40 hours. The insoluble mucoprotein fraction was washed repeatedly with water acidified with HCl to pH 2.3. It was then washed with ethanol and ether and dried in vacuo over P_2O_5.

The total yield of material after peptic digestion was less than 40%, indicating that the carbohydrate constituents probably make up less than half of the total solids present in pooled sputum, the remainder of the components being either proteins or nucleoproteins.

Brogan has isolated two mucopolysaccharide fractions, one soluble and one insoluble in a 90% (w/v) phenol-water mixture (Morgan & King, 1943) and also an electrophoretically homogeneous mucoprotein from bronchial mucus. The mucoprotein was found to have some properties in common with the influenza antihemagglutination mucoprotein isolated from urine. The phenol insoluble mucopolysaccharide was isolated by the same technique as devised by Morgan & King for the separation of blood group A substance.

The protein material split by the pepsin digestion was not studied by Brogan. The bulk of these proteins most probably originates from the cellular material present in the sputum. There is as yet no satisfactory method for isolating the cellular material from the sputum without subjecting the cells to some degree of damage.

Coagulum formation of the bronchial secretion is a disturbing factor in the examination of the exfoliated cells. Some authorities have advocated a careful and painstaking selection of the whitish areas or blood flecks rich in cells for the preparation of cytological slides (Nieburgs, 1956; Jackson, Bertoli, Ackerman, 1951).

Some authors have used proteolytic enzymes for liquefaction of the sputum and liberation of the cells from the mucoprotein-mucopolysaccharide coagulum. Cell preservation is

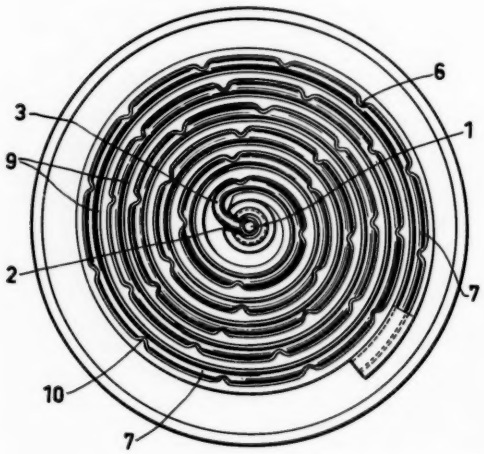

Figure 3: Type of rotor used in this investigation, 1; tubular inlet; 2, slot in the tubular shaft of the centrifuge; 3, plastic tubing; 6, ridges or thresholds on the peripheral wall of the spiral groove 9; 7, intercommunicating shallow compartments in the tubing; 10, constrictions on the peripheral wall of the tubing. In threshold centrifuging dissolved mucus, the heavier intact cells and cell clusters will be found in the central coils, and bacteria which form a semitransparent film together with some detritus in the peripheral coils. Note: some bacteria may escape from the peripheral opening of the tubing 3, if the R.C.F. is too low in the peripheral coils or if the suspension is run too fast through the tubing. After stopping the centrifuge, the tubing is cut into sections corresponding to one or several compartments and the cells and bacteria are exposed by cutting these pieces longitudinally.

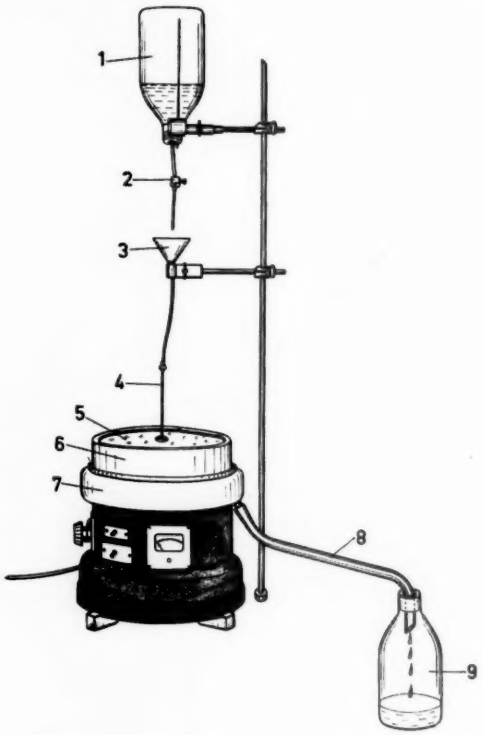

Figure 4: Perspective view of the threshold centrifuge used in this investigation. 1, bottle containing the dissolved mucus; 2, drop regulator; 3, funnel which can be used with or without a filter; 4, thin stainless steel tube leading the suspension to the central inlet of the rotor shown in Fig. 3; 5, plexiglass window of the removable centrifuge lid; 6, centrifuge lid of stainless steel; 7, collector of stainless steel; 8, outlet tubing conveying the suspending agent from the collector to the bottle 9.

sometimes good, but frequently both the cells and the mucus are digested by the proteolytic enzymes. Some laboratories have utilized "Turmix" apparatus for the liberation of cells from the mucus. Some authors have developed cytohistologic methods which involve paraffin embedding for the examination of the sputum cells (1, 24, 25).

The present investigation:

The present paper is a preliminary report on the liquefaction of the bronchial mucus by treatment in an excess amount of physiological saline at 37° C in the presence of hydrogen peroxide, and the subsequent separation and concentration of the cells and the bacteria by means of the threshold centrifuge.

Method:

The patients are asked to expectorate sputum into 1-liter bottles containing 500 ml of sterile saline solution. They are instructed to remove food residues by washing the mouth. The amount of oral and nasopharyngeal cells can be limited if the patient washes the oral cavity between expectorations. The sputum is thus transferred immediately into an isotonic milieu. The patients are asked to shake the bottles and see that the sputum does not adhere to the wall of the upper part of the bottles without falling into the isotonic salt solution. The sputum collection is begun in the morning and the bottles sent to the laboratory, as soon as a sufficient amount of sputum has been produced.

Liquefaction of the sputum coagulum:

The bottles are placed in a water bath at 37° C and the contents are stirred gently. A considerable fraction of the sputum is liquefied at 37° C. However, upon cooling the contents of the bottles, this liquefied fraction is reprecipitated. When hydrogen peroxide, 2 ml 10% solution, is added to the warmed suspension, the mucus dissolves completely and is not recoagulated on cooling. Hydrogen peroxide is added, 2 ml at a time while the flask is stirred, until all of the sputum is dissolved. The fact that hydrogen peroxide liquefies the sputum at body temperature and not at room temperature under similar conditions points to some enzymic reaction. The cells embedded in the coagulum are thus liberated from the bulk of the sputum. Large leukocyte conglomerates, pieces of necrotic tissue, and other cells exfoliated in groups or clusters are not divided into single cells by this procedure. This kind of treatment with low concentrations of hydrogen peroxide does not seem to affect the microscopic appearance of the cells.

If the suspending agent gets too viscous after dissolving the mucoprotein-mucopolysaccharides, the volume should be increased to 1 or 1.5 liters with physiological saline before centrifuging. This dilution will, at the same time, decrease the specific gravity of the colloid solution and facilitate the sedimentation of the cells and the bacteria.

The dissolved mucoprotein and mucopolysaccharides can be precipitated by lowering the pH with concentrated acetic acid or dilute hydrochloric acid.

The threshold centrifugation of the cell suspension:

After liquefaction of the mucus, the suspension is centrifuged continuously through a spiral model of the threshold centrifuge originally constructed by one of us (Rastgeldi, 1957) (Fig. 3). Running at a speed of 4000 r.p.m., the centrifuge gives a relative centrifugal force of c:a 200 × G in the central coils and 1000–1200 × G in the coils at the periphery of the rotor. Thus, the heavier single cells and cell conglomerates are deposited at the beginning of the sedimentation area where the relative centrifugal force is lowest, whereas the lighter particles, e.g., cell remnants, detritus, bacteria, etc., are continually carried towards the periphery of the rotor where they are subjected to a much higher relative centrifugal force and progressively precipitated. The portion of the rotating channel between the deposit of heavy intact cells centrally and the deposit of bacteria peripherally, contains a mixed population of free nuclei, cell remnants, detritus, etc. It has less cytological and bacteriological value and can be discarded. One, or at most two, slides from the beginning of the sedimentation area will give a satisfactory cytological picture. A corresponding, clear bacteriological picture will be found at the peripheral end of the rotating channel.

Bacteria ingested by leukocytes, macrophages, "heart failure cells," etc. appear intracellularly at the beginning of the sedimentation area. Sometimes groups of bacteria appear intracellularly in the nonphagocytizing cells, e.g., the epithelial cells. This indicates the penetrating power of some strains of bacteria which probably use the cytoplasm as a substrate.

Comparison of the centrifuged smears with direct smears:

Both direct slides and the threshold-centrifuged ones were fixed immediately in 95% alcohol-ether, 1/1 mixture and stained according to the Papanicolaou technique. The threshold-centrifuged cytological slides had a large concentration of intact cells with a clear background, whereas the direct smear slides contained a few cells mixed with cell remnants, detritus, free nuclei, bacteria, etc. Further, the cells in the direct smears were embedded in mucus which obscures the boundary between the cell membrane and the mucus, and the boundary between the nuclei and the cytoplasm. In the direct smear technique it is sometimes difficult to differentiate the extracellular bacteria from intracellular bacteria, as the microorganisms can lie above or below the cells, whereas in the centrifuged smears the extracellular bacteria are washed away together with other small particles.

Bacteriological slides were prepared from the peripheral section of the rotating channel and stained by the Gram technique. Tuberculous material has been excluded from this investigation because of the risk of infection. (See text for figures.)

Discussion:

Although cytology is gaining widespread acceptance, especially in the diagnosis of cancer, there is as yet no satisfactory method for isolating a large number of diagnostically valuable cells from the bulk of the bronchial mucus. This has been due to two main reasons: (1) Lack of a method for liberating the embedded cells from the mucoprotein-mucopolysaccharide coagulum without at the same time subjecting the cells to some degree of damage. (2) Lack of efficient and practical means for the concentration of the liberated cells by separating them from cell remnants, detritus, bacteria, etc. The present investigation shows that these difficulties can be overcome to satisfaction.

Previously, it has been shown by us that the intact cells do not lose their cytological identity by threshold centrifugation. It has been suggested that normal erythrocytes can be used as a criterion as to the reliability of the suspending agent, in this investigation physiological saline with hydrogen peroxide at 37° C. No hemolysis should occur in the clear suspending agent ejected from the threshold centrifuge, at least within a period of time corresponding to the time elapsed between the beginning of the collection of mucus and the end of centrifugation.

In fact, the erythrocytes treated in physiological saline at 37° C in the presence of hydrogen peroxide and then centrifuged and stained in the same way as the sputum, gave very vivid and clear slides.

In chronic bronchitis and bronchiectasis the cells found in the sputum are predominantly leukocytes, usually in groups or clusters. It is very difficult to make satisfactory smears of the concentrated bronchial leukocytes. A histocytological method can be applied whenever it is difficult to distribute the cells satisfactorily on a slide.

Figure 5a: Direct smear. Cells obscured by mucus. ×150.

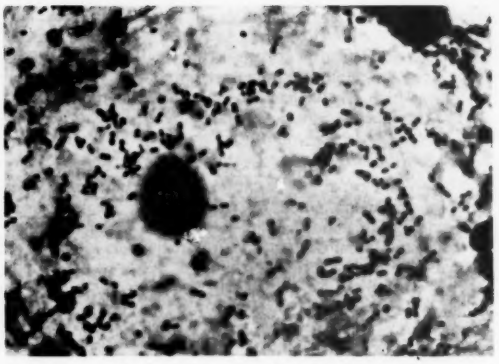

Figure 5d: Centrifuged smear. Peripheral part. Large number of diplococci. ×1500.

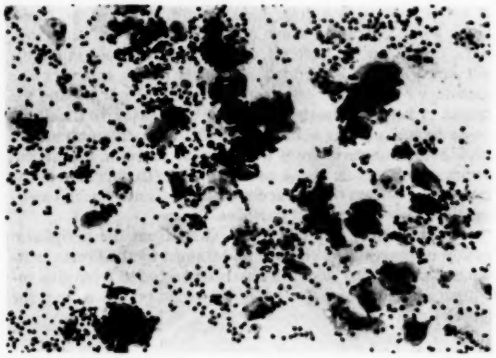

Figure 5b: Centrifuged smear. Central part. Large number of intact cells liberated from mucus. ×150.

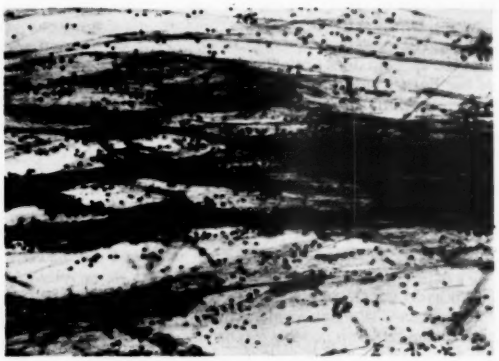

Figure 6a: Direct smear. Heavy mucus embedding the cells. ×150.

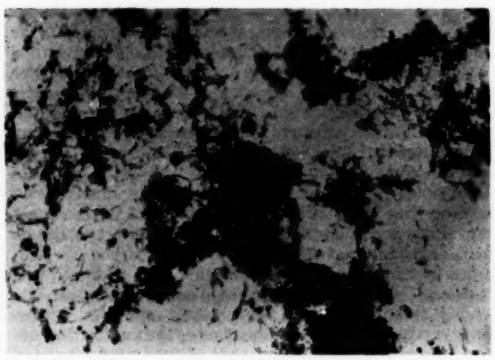

Figure 5c: Centrifuged smear. Peripheral part. Cell remnants and detritus separated from intact cells. ×150.

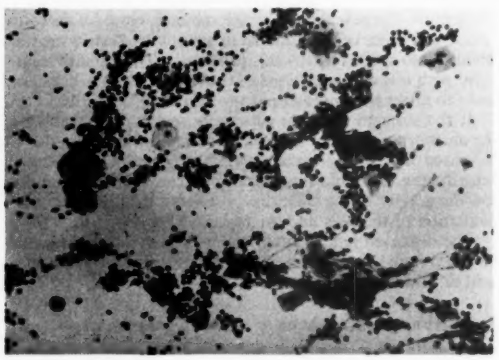

Figure 6b: Centrifuged smear. Central part. A concentration of intact cells liberated from mucus. ×150.

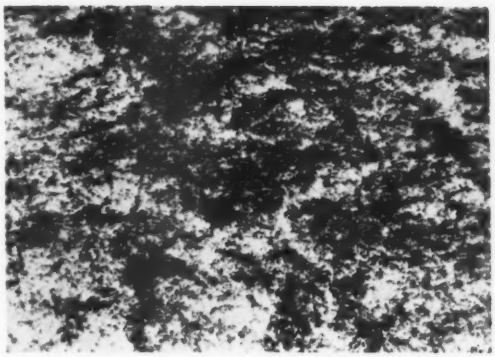

Figure 6c: Centrifuged smear. Peripheral part. Cell remnants and detritus separated from intact cells. ×150.

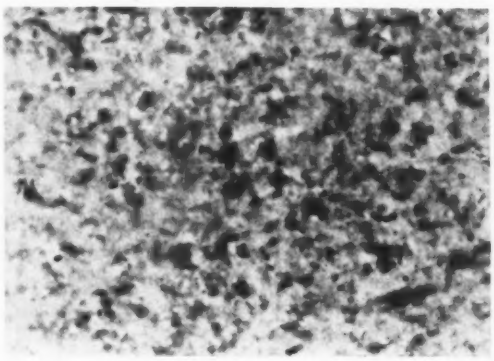

Figure 6d: Centrifuged smear. Some bacteria on a background of detritus. ×1500.

Figure 7: A squamous epithelial cell invaded by bacteria. Extracellular bacteria have been washed to the peripheral part of the sedimentation area leaving the intracellularbacteria at the central part. ×1500.

Summary:

1. A method for the liberation of the cells from the bronchial mucus is described.
2. The intact cells liberated from the mucoprotein-mucopolysaccharide coagulum have been concentrated by threshold centrifugation.
3. The bacteria have been simultaneously concentrated by means of the threshold centrifugation.

Acknowledgements.

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We are indebted to Dr. Hannelore Ostberg and Mrs. Elisabet Bylund for the critical examination of the slides, to Miss Anni Andersson for staining the cytological slides and to Miss Maj-Lis Kain for staining the bacteriological slides.

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THE SYMPOSIA BY CORRESPONDENCE OF ACTA CYTOLOGICA

INTRODUCTORY REMARKS

The Symposia of ACTA CYTOLOGICA are *held entirely by correspondence* and contain international discussions of scientific problems of interest to the exfoliative cytologist.

System for Selecting Subjects for Symposia: From recommendations received, the Editorial Office will draw up the list of subjects and will publish these subjects in ACTA CYTOLOGICA, under the heading FUTURE SYMPOSIA.

The final detailed program will be published in ACTA CYTOLOGICA immediately preceding the one where these topics are to be considered, under the heading, THE NEXT SYMPOSIUM.

Instructions for Authors: Each problem will be introduced by a *Main Speaker* or *Speakers*. These principal papers will then be considered by persons identified as *Discussants*. As a general rule, approximately 600 words each will be allocated for main papers and 200 words each will be allocated for the contributions of the Discussants. The Main Speakers will then be given the opportunity to make unlimited Closing Remarks.

Photomicrographs and tables may be reproduced: one full page for each principal paper and for the paper of the Discussant (maximum one-half page per contribution). The photomicrographs and tables should be submitted in glossy photographic prints, preferably in the size of 3 × 4 inches (i.e., 12 × 19 cm) and should show a proportional 10μ scale on its reverse side. *Each figure should be accompanied by a comprehensive caption.*

The Discussants are requested *to strictly restrict their contributions to the discussion of the main papers.* Discussions which are not directly related to the main paper cannot be accepted. It is suggested that the Discussants prepare their contributions in such a manner that the reader may gain the impression of an actual round table conference.

The Closing Remarks of the Main Speakers should be limited to the answering of questions raised in the discussion and to other directly related information.

The Bibliography for the papers of both Main Speaker and Discussant should be organized in the same manner as in the American Journal of Obstetrics and Gynecology, at the *end* of the paper. *Every cited opinion or publication should have a reference in the bibliography.*

Deadline for Contributions: The Editorial Office will set deadlines for each written symposium. These will include:

1. deadline for agreements to contribute.
2. deadline for main papers.
3. deadline for discussions.
4. deadline for closing remarks.

Reprints: Authors may receive reprints of their papers by ordering these reprints before the particular issue goes to press. There will be a nominal charge for reprints: $6.00 per page for the first one hundred copies, and $3.00 per page for each additional hundred.

LES SYMPOSIA PAR CORRESPONDANCE DES ACTA CYTOLOGICA

Les *Symposia par Correspondance* des ACTA CYTOLOGICA présentent des discussions internationales sur des problèmes scientifiques intéressant le cytologiste exfoliative.

Système du choix des sujets pour les sympposia: En partant des propositions, et sous la rubrique: FUTURS SYMPOSIA, le bureau de rédaction dressera la liste des sujets principaux qui seront publiés dans les ACTA CYTOLOGICA.

Le bureau de rédaction établira le programme définitif et détaillé des discussions qui sera publié dans les ACTA CYTOLOGICA précédant immédiatement le symposium, sous la rubrique PROCHAIN SYMPOSIUM.

Recommandations pour les auteurs: Chaque sujet principal sera présenté par un Rapporteur Général ou des Rapporteurs. Ces mémoires principaux seront alors soumis aux Participants à la Discussion. En règle générale 600 mots seront accordés aux Rapporteurs des sujets principaux, et, 200 mots aux Participants à la Discussion. Les Rapporteurs Généraux pourront clôturer les discussions par un nombre illimité de remarques.

Des microphotos et graphiques pourront être reproduits à raison d'une page entière pour chaque sujet principal et une demi page au maximum pour les discussions. Les microphotos et les graphiques doivent être présentés sur du papier brillant, de préference dans le format 12 × 19 cm. *Chaque figure devra être accompagnée d'une legende explicative précise.*

Les membres et invités prenant part aux discussions sont invités à *limiter strictement leurs interventions aux discussions des sujets principaux.* Des discussions qui n'ont pas de rapport direct avec le sujet principal *ne pourront être acceptées.* Il est recommandé que les discussions soient rédigées d'une manière telle que le lecteure a't l'impression d'assister à une discution réelle de table ronde.

Les Remarques de Clôture du Rapporteur Général devront se limiter à la réponse aux questions soulevées dans les discussions et aux autres informations éventuelles ayant un rapport direct avec le sujet.

La bibliographie des rapports et discussions devra être rédigée de la même manière que celle de l'American Journal of Obstetrics & Gynecology et figurer à *la fin* du texte. *Chaque opinion ou publication citée dans le texte doit avoir sa réference dans la bibliographie.*

Dates limite pour les collaborations: Le bureau de rédaction, fixera des dates limites comprenaet:

1. un délai pour l'acceptation des collaborations,
2. un délai pour les sujets principaux,
3. un délai pour les discussions,
4. un délai pour les remarques de clôture.

Tirés-à-part: les auteurs pourront obtenir des tirés-à-part de leurs communications en les demandant avant la mise sous presse des ACTA CYTOLOGICA publiant leurs articles. Les tirés-à-part seront facturés: $6.00 par page de texte pour le premier cent et $3.00 pour chaque centaine supplémentaire.

DIE SCHRIFTLICHEN SYMPOSIEN DER ACTA CYTOLOGICA

Die schriftlichen Symposien der ACTA CYTOLOGICA befassen sich auf internationaler Basis mit wissenschaftlichen Problemen, die für den Exfoliativ-Zytologen von Interesse sind.

System der Thema-Auswahl für die Symposien: Die Schriftleitung stellt auf Grund von Thema-Vorschlägen eine Liste von Haupt-Themen zusammen, und gibt diese Liste unter dem Titel ZUKÜNFTIGE SYMPOSIEN bekannt.

Die Schriftleitung bereitet das Programm mit allen Einzelpunkten vor, und veröffentlicht dieses Programm in dem Heft, das dem betreffenden Symposion vorausgeht, unter dem Titel DAS NÄCHSTE SYMPOSION.

Instruktionen fur Autoren: Jedes Thema wird von einem oder mehreren Referenten behandelt. Diese Referate werden dann von Diskussions-Vortragenden besprochen. Im allgemeinen werden Referate auf etwa 600 Worte beschränkt, und Diskussions-Vorträge auf 200 Worte. Die Referenten erhalten dann die Gelegenheit, Schlussbemerkungen ohne Wortzahlbeschränkung zu machen.

Mikrophotographien und Tabellen können abgedruckt werden: eine Ganzseite kann Referenten und eine halbe Seite Diskussionsvortragenden für Abbildungen zur Verfügung gestellt werden. Die Photographien sind auf Hochglanzpapier, und möglichst in der Grösse 12 × 19 cm erbeten und soll ein proportionales 10μ Zeichen auf der Rückseite haben. *Jede Abbildung muss von einem erklärenden Untertitel begleitet sein.*

Die Diskussionsvortragenden sind gebeten, sich in ihren Beiträgen *streng an das Hauptreferat zu halten.* Diskussionsbeiträge, die sich nicht an das Hauptthema halten, *können nicht berücksichtigt werden.* Es wird vorgeschlagen, dass die Diskussionsvorträge in einem Stil abgefasst sind, dass der Leser den Eindruck gewinnt, als ob es sich um eine Diskussion am runden Tisch gehandelt hätte.

Die Schlussbemerkungen der Referenten sollen sich nach Möglichkeit auf die Beantwortung von Diskussionsfragen beschränken.

Die Bibliographie der Referate und der Diskussions-Vortäge sollen *am Schluss* der Beiträge nach dem Muster der Bibliographien im American Journal of Obstetrics and Gynecology aufgeführt werden. *Jede zitierte Ansicht oder Publikation muss eine Referenz in der Bibliographie haben.*

Termine für Beiträge: Die Schriftleitung setzt Termine für die Schriftlichen Symposien fest. Die folgenden Termine werden bekanntgegeben:

1. Termin für Erhalt der Beitrags-Zusagen,
2. Termin für Erhalt der Hauptreferate,
3. Termin für Erhalt der Diskussions-beiträge.
4. Termin für Erhalt der Schlussbemerkungen.

Sonderdrucke: Autoren können Sonderdrucke ihrer Beiträge bestellen, bevor die betreffende Ausgabe in Druck geht. Die Schriftleitung muss diese Sonderdrucke berechnen und wird einen Betrag von $6.00 pro Seite und 100 Sonderdrucke, und einen Betrag von $3.00 für jedes weitere Hundert erheben müssen.

SIMPOSIUM ESCRITO DE ACTA CYTOLOGICA

El simposium escrito de ACTA CYTOLOGICA contiene discusiones internacionales sobre problemas científicos que son de interés para el citólogo exfoliativo.

Sistema de selección de materias para el simposium: Con sugestiones recibidas, la oficina editorial confeccionará una lista de los temas más interesantes, lista que será publicada en ACTA CYTOLOGICA con dos numeros de anticipación a la fecha de su posible publicación, bajo el epígrafe de "SIMPOSIUM FUTUROS."

La Oficina Editorial confeccionará y publicará una lista detallada del programa de la discusión en el número de ACTA CYTOLOGICA immediatamente anterior a aquel en que han de ser incluidos los temas, bajo el epígrafe de: EL PROXIMO SIMPOSIUM.

Participación en el Simposium Escrito: No habrá restricción alguna sobre el número de puntos de discusión en los que cualquier autor desee participar.

Instrucciones a los Autores: Cada problema deberá ser presentado por un ponente o ponentes. Estos trabajos principales serán entonces discutidos por los comunicantes. Como regla general, se permite un máximo de 600 palabras para los trabajos principales y 200 palabras para las contribuciones de los comunicantes. Al ponente principal se le da la oportunidad de hacer rectificaciones finales ilimitadas.

Pueden reproducirse microfotografías y tablas: una página por cada trabajo principal y un máximo de media página por discusion. Las microfotografías y tablas deberán enviarse en forma de copias fotográficas amplias. A ser posible de 3 × 4 pulgadas (12 × 19 cms). *Cada figura deberá acompañarse de su correspondiente leyenda.*

Se suplica a los comunicantes *ajustar estrictamente sus comunicaciones a la discusión de los trabajos principales.* Las discusiones que no estén directamente relacionadas con el trabajo principal *no podrán ser aceptadas.* Se sugiere que los comunicantes realicen sus contribuciones de manera tal que el lector tenga la impresión de estar ante una verdadera mesa redonda.

Las rectificaciones finales de los ponentes deberán limitarse a contestar las preguntas aparecidas a lo largo de la discusión asi como a otras directamente relacionadas con el tema.

La bibliografia, tanto de las ponencias como de las comunicaciones deberá redactarse de la misma forma que figura en el American Journal of Obstetrics and Gynecology, *al final* del trabajo. *Toda op'nión o publicación citada deberá tener su correspondiente referencia en la bibliografía.*

Fechas para las contribuciones: La Oficina Editorial, fijará fechas límite absolutas para cada simposium escrito. Estas incluirán:

1°. Fecha límite para acuerdo de contribución,
2°. Fecha límite para las ponencias,
3°. Fecha límite para las discusiones,
4°. Fecha límite para las anotaciones finales.

PROGRAM OF SYMPOSIUM OF VOL. III, NO. 2, 1959

HORMONAL CYTOLOGY DURING PREGNANCY AND THE POSTPARTUM PERIOD

Symposium B

HORMONAL CYTOLOGY DURING PREGNANCY AND THE POSTPARTUM PERIOD

THE RATE OF PRODUCTION OF ENDOGENOUS HORMONES IN PREGNANCY

JOSEPH ZANDER
Cologne, Germany

For understanding and judgment of hormonal cytology during pregnancy, knowledge of not only the quality but also the quantity of the hormones produced is important. By application of newer methods our knowledge in this regard has broadened. The following is a short summary of this knowledge.

A. Which hormones are produced in the placenta during pregnancy?

As known for a long time, the trophoblast already produces chorionic gonadotrophin a few days after implantation of the egg. More recent findings favor the idea that the production of steroids also starts during the first month of pregnancy (13). Thus, the continuation of the pregnancy is also possible in cases where the corpus luteum graviditatis is removed shortly after completion of the first month of pregnancy. Placentas from the second month of pregnancy contain gestational substances in higher concentrations than in the following months (13). The pregnancy-preserving function of the corpus luteum graviditatis has certainly been overestimated in former times in respect to the duration of this function.

So far there is no information regarding the ranges of the production of chorionic gonadotropin. It is most likely, however, that the well-known peak of chorionic gonadotropin excretion, between the second and third month of pregnancy, is an expression of increased hormonal production. The decreased excretion of chorionic gonadotropin in the latter periods of pregnancy is not the result of an increased consumption of hormones, as previously assumed, but the result of decreased production.

Whether or not the placenta forms corticosteroids and androgens in considerable amounts is still uncertain. Corticosteroids and androstenedione are found in the placenta. However, that does not necessarily mean that they are formed in the placenta. On the other hand, we have enough proof that estrogens and gestational substances are produced by the placenta.

B. How can the amount of endogenously formed hormones be found?

The simplest method is to supplement the normal function of the organ in question by artificially adding the hormones needed. Thereby, the same situation will be established which is otherwise provided by the normal activity of the gland. Once the correct amount of hormone is found in this way, one can assume that under physiological conditions the same amount is produced by the gland in question. In regard to pregnancy, this approach has been applied by Bradbury and associates(1, 7). They produced some type of pseudo-pregnancy in women who previously menstruated normally or were ovariectomized. Thus, they could find which dosage of progesterone leads to the formation of a predecidual condition of the endometrium in ovariectomized women. If estrogens are given simultaneously, this dosage is approximately 100 mg per day.

Another method is based upon the excretion of metabolites of the respective hormones under physiological conditions. If the same hormone is injected, preferably in an isotope-marked form, one can tell what per cent of this hormone is excreted in metabolized form with the urine. Suppose that the endogenously-produced hormone is metabolized the same way at the same rate as the injected one. Then the production rate of the hormone can easily be computed.

The third method is the most accurate. The concentration difference of the hormone in the arterial and venous vessels of the organ is measured, as well as the flow volume in those vessels, and from these values the amount of hormone produced by the gland is computed.

C. What amounts of gestational and estrogenic substances are formed during pregnancy?

Gestagens: Progesterone was injected in varying dosages (10) to climacteric women. Subsequently the percentage of the pregnandiol excreted in the urine was determined. A pregnandiol excretion, comparable to that of the first half of the pregnancy, was reached when 25-50 mg of progesterone was given to the patient per day. By administering 100 mg progesterone per day, the excretion rate of the second half of pregnancy could be provoked.

In more recent studies (12), we measured the hormonal concentration directly from the vessels by draining the placenta, the umbilical vessels and the uterine veins. By application of the known values for the volume per minute in the maternal and fetal placental vessels, we could compute that, at the end of the pregnancy, about 190-280 mg progesterone per day reach the maternal organism across the placental site through the venous uterine blood (11, 13). About 75 mg go into the fetal organism with the cord blood.

Pearlman (8) computed the progesterone production in the range of 250 mg per day at the end of the pregnancy. His calculations were based upon examinations of the dilution of isotope-marked progesterone administered and determination of the specific activity of pregnandiol in the urine.

Accordingly, it can be assumed that the progesterone production during pregnancy increases from about 25 mg in the beginning to about 250-300 mg in the late pregnancy in a probably continuous way.

Estrogens: The estrone and estradiol excretion during pregnancy increase up to one hundred times as compared to the normal cycle; the excretion of estriol even increases up to one hundred times (4). Thus, it is most likely that in addition to an increased production of gestational substances during pregnancy, we also encounter an increased production of estrogenic hormones.

The cause of the special increase of the estriol excretion was not quite understood until recently. Two possibilities are under discussion: either the placenta releases increased estriol or we deal with an increased, extra placentar conversion of estrone-estradiol to estriol, for example the conversion by the liver during pregnancy.

At the present time we lean more towards the first interpretation. In the placenta more estriol than estradiol (6) can be found. According to our own findings (2), practically only estrone and estradiol are found in the corpus luteum menstruationis and in the ripe Graafian follicle. Estriol could hardly, or not, be demonstrated. This corresponds with the fact that, during the menstrual cycle, as compared to pregnancy, only relatively small amounts of estriol are excreted (3). It can be interpreted, therefore, that the high estriol content of the placenta is a result of a true increase of production in this organ. According to Ryan (9), the estriol production in the placenta is not possible only via estrone-estradiol production. The estriol can be synthesized directly from a non-estrogenic precourse.

Brown (5) recently performed excretion experiments to clarify the question of estrogen production in pregnancy. He found very constant values in a number of patients, and thus his figures give a fairly good basis for the calculation of estrogen production.

If he injected estrone or estradiol to his patients (menopausal or amenorrheic women), he recovered a total of 9% in the form of estrone and estradiol and 7% in the form of estriol in the urine. In cases where he injected estriol, he recovered 56% of the dose, exclusively estriol. The relation of the three excretion forms during the menstrual cycle corresponds almost exactly to the excretions after parenteral administration of estrone-estradiol. Consequently, it can easily be calculated that during the cycle between 0.08 and 0.34 mg per day estrone-estradiol are produced, depending upon the phase. During pregnancy, the calculation of the estrogen production is more difficult and can only be undertaken under supposition that estriol, besides estrone and estradiol, enters the blood stream in rather high amounts directly from the placenta. Under this assumption, the amount of estrone, estradiol and estriol produced by the placenta increases from 1 mg per day in the tenth week of pregnancy to 50-100 mg per day at the end of pregnancy. Up to the seventh week, the placenta does not produce any additional estriol. In the

twentieth week about 42%, in the thirtieth week, about 53% and at the end of pregnancy about 70% of the total estrogens formed by the placenta are estriol.

The above values inform us of the general magnitude of the hormones produced during pregnancy. It seems necessary to offer further evidence, especially where the estrogens are concerned. I also believe that cytology can contribute to this important question, which is not merely of theoretical interest.

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DISCUSSION

E. JÜRGEN PLOTZ, Chicago, Illinois, U.S.A.:

As Zander has pointed out, there is strong evidence that the placenta produces large amounts of estrogens in human pregnancy. Our own experiments using C^{14}-1-acetate as substrate demonstrated the synthesis of estrone from this compound by human placental tissue slices (1).

Pearlman and co-workers (2), using homogenates of placental tissue, and Ryan and Angel (3), using tissue slices, demonstrated the ability of the human placenta to convert estradiol to estrone. Moreover, Meyer (4) reported the conversion of androstenedione to estrone in in-vitro experiments.

Similar in-vitro experiments (5) demonstrated almost conclusively that the placenta is capable of producing progesterone (6). Although it appears to be well established that the placenta is the source of estrogens and progesterone, there is no evidence that androgenic hormones are produced by this organ.

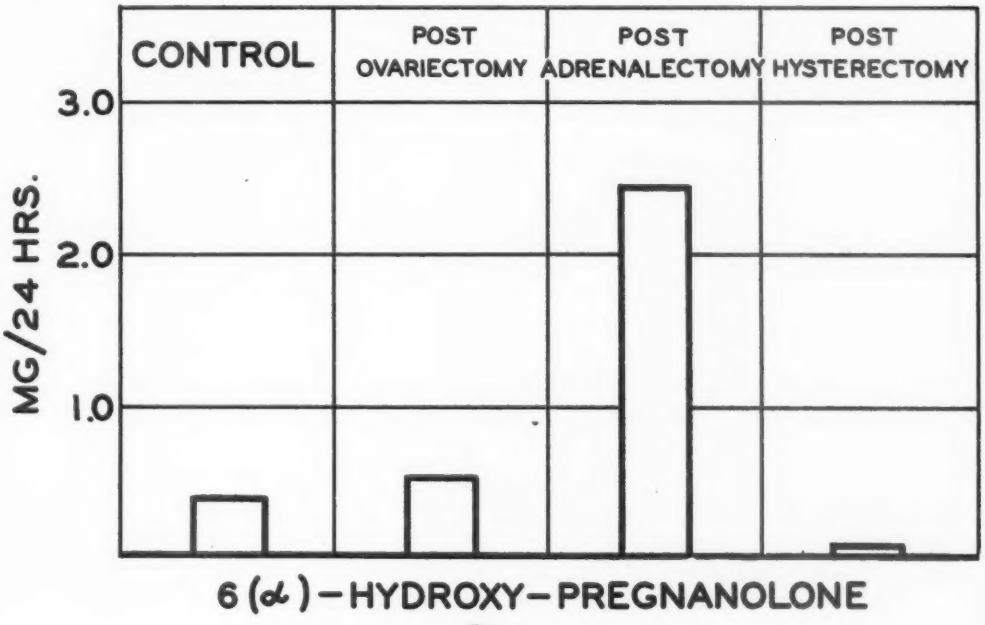

Fig. 1

It is known that certain urinary metabolites of androgens, such as androsterone and etiocholanolone, decrease to low values with advancing pregnancy (7). In some instances no androsterone can be detected in human pregnancy urine obtained during the third trimester (8).

Recent studies have indicated that placental tissue is capable of adding an oxygen function to the progesterone molecule. When term or near term placentas were perfused with bovine blood containing radioactive progesterone, tagged 6-keto-progesterone was isolated from the perfusate (9). This observation is of interest in the light of an observation in our own laboratory. There was a marked post-operative rise of the urinary excretion of 6-hydroxypregnanolone in a pregnant woman who had been subjected to bilateral ovariectomy and adrenalectomy because of recurrent cancer of the breast (Fig. 1). The recent study of Berliner and Salhanick (10) demonstrates the presence of a 6-(β)-hydroxylase in the human placenta. These findings indicate that the placenta becomes adapted under certain experimental and pathological conditions so that oxygen can be added to progesterone at position 6. The biological significance of 6-(α or β)-hydroxylated C^{21}-steroids is not established to date.

Zander has given a complete and brilliantly interpreted summary of the present knowledge of the quantitative agents of hormonal production by the human placenta.

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CLOSING REMARKS

JOSEF ZANDER:

In addition to Plotz' interesting remarks regarding the 6-oxygenating systems in the human placenta, a recent result of our laboratory may be of interest. We could demonstrate the presence of 6-(β)-hydrozylase in a human ovary under certain pathological conditions.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

HORMONAL BASIS OF THE CYTOLOGICAL CHANGES DURING PATHOLOGICAL PREGNANCIES

OTTO STAMM, V. RAWYLER AND GUSTAVE RIOTTON
Geneva, Switzerland

It has been considered that in the absence of local vaginal mitation or infection, a deviation in the Eosinophilic (E. I.) and Karyopyknotic (K. I.) Indices during pregnancy is the manifestation of a hormonal disturbance. Until now an increase of the Eosinophilic Index was attributed to a decrease of either relative or absolute progesterone activity. However, the comparison of smears during pregnancy with simultaneous determinations of pregnandiol and estriol, as well as the experimental reproduction of these cytological changes in castrated women, presents an argument against this theory.

Material:

Fifty-three cases of late threatened abortion (T. A.) (16th to 27th week) and threatened premature labor (T. P. L.) (28th to 37th week).

Smears: before the onset of hormonal treatment, by aspiration in the posterior fornix, stained according to Papanicolaou.

Hormone determinations: pregnandiol (method of Huber, Borth and de Watteville); estriol (method of Brown).

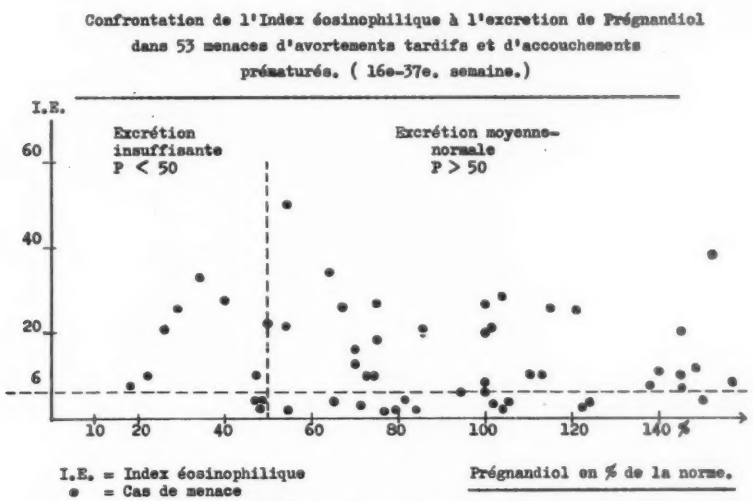

Fig. 1. Comparison of Eosinophilic Index and pregnandiol excretion in 53 cases of threatened abortion (16-37th week).

Results:

1) Cyto-hormonal evaluation of progesterone secretion

In 53 cases of T. A. and T. P. L., the instance of pathological smears (E. I. above 6) is approximately the same for the cases with (73%) or without (64%) luteal insufficiency (Fig. 1). One cannot, therefore, interpret an increase in E. I. alone as an indication of either relative or absolute luteal insufficiency.

2) Cyto-hormonal evaluation of estrogen secretion

Smears are rarely pathological (20% of the cases) with an intense estrogen stimulation, whereas the E. I. is very frequently increased when the estriol excretion is 45% below the normal (Fig. 2). There would seem to exist, therefore, a marked relationship between the estrogen stimulation and the E. I. and K. I.; a low estriol excretion corresponding generally to high E. I. and K. I., and vice versa.

Confrontation de l'Index éosinophilique à l'excrétion d'Oestriol dans 19 menaces d'avortements tardifs et d'accouchements prématurés. (16e-37e. semaine)

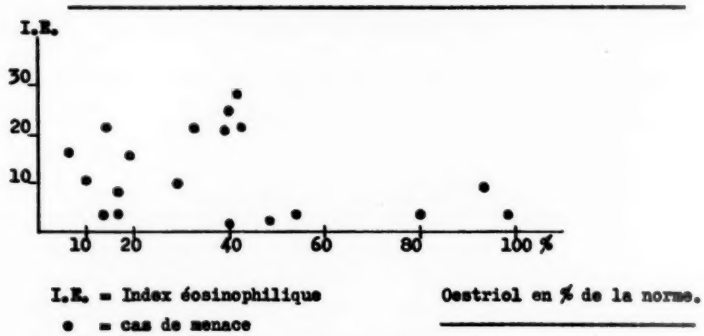

Fig. 2. Comparison of Eosinophilic Index and estriol excretion in 19 cases of threatened abortion (16-37th week).

3) Cyto-hormonal evaluation of progestogen-estrogen imbalance

Figure 3 shows that the majority of cases with pathological smears have an estrogen insufficiency without luteal insufficiency, whereas the cases of threatened abortion with normal cytology usually have a normal level of estriol. Estrogen insufficiency would thus seem to be the principal cause of increased E. I. and an intense estrogen stimulation in the presence of progesterone (average levels of 50% or more of normal) would seem to be an essential requirement for a smear showing normal pregnancy cytology.

Corrélation entre cytologie vaginale et situation hormonale (oestrogènes et progestérone) dans 19 menaces (16-37 semaines.

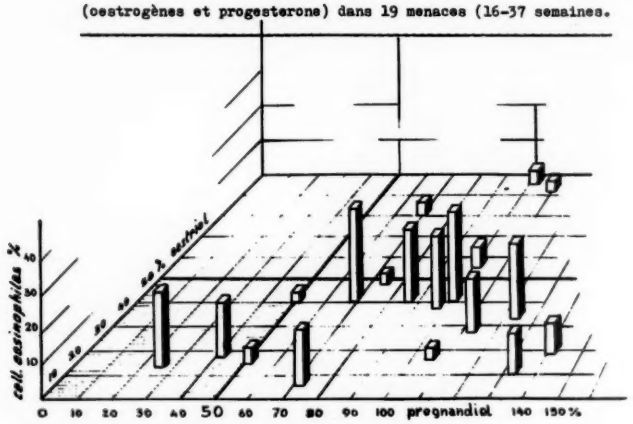

Fig. 3. Comparison of Eosinophilic Index and excretion of estriol and pregnandiol in 19 cases of threatened abortion (16-37th week).

4) Experimental confirmation

We have been able to verify experimentally that an intense estrogen stimulation is the essential condition producing a pregnancy-type smear and that a decrease in the estrogen level can be the cause of an increased E.I. (3). In fact, we reproduced typical smears of pregnancy in castrated women by increasing doses of estrogens (Fig. 4). After complete withdrawal of estrogen medication, we obtained typical smears of slight and then severe threatened abortion, with elevation of E.I. and K.I. However, the estrogen treatment must be preceded by injection of a long acting androgen (Perandrene cristallin-Ciba).

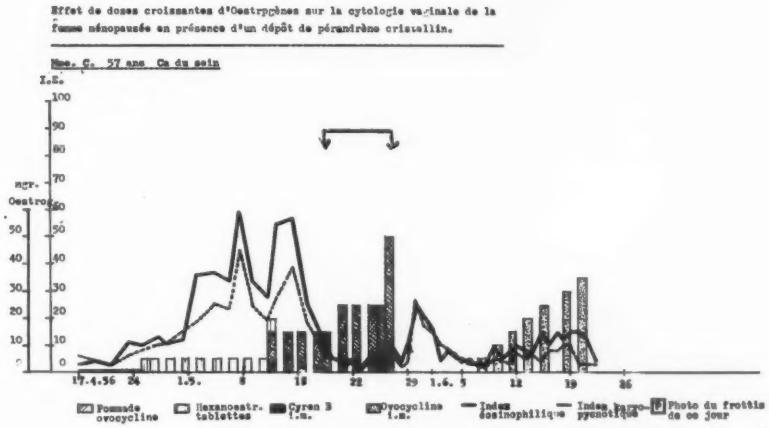

Fig. 4. Experimental study on the behavior of Eosinophilic and Karyopyknotic Indices in castrated women treated with increasing doses of estrogens. Period with typical pregnancy smear. After withdrawal of estrogen medication, apparition of an elevation of Eosinophilic and Karyopyknotic Indices with vaginal smears, typical for severe threatened abortion.

Conclusion:

On the basis of clinical findings and hormonal determinations, we have shown that an elevation of the Eosinophilic Index during pregnancy usually reflects an insufficient estrogen stimulation. This opinion is supported by an experimental study on castrated women in whom we have been able to produce elevated Eosinophilic and Karyopyknotic Indices by decreasing the estrogen dosage.

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EPITHELIAL AND CONNECTIVE TISSUE MODIFICATIONS DURING PREGNANCY

JEAN A. de BRUX
Paris, France

The effects of gestation on epithelial and connective tissues are relatively well-known, but their causes and the mechanism of their action remains as yet obscure. Several factors, in fact, influence these changes:

(a) the maternal hormones < pituitary, ovarian, adrenal

(b) the placental hormones, still poorly defined

(c) the fetal hormones (which play a supplementary or additional role).

These different hormones are so closely interrelated in their action that it is difficult to determine precisely the individual part played by any one of them.

Furthermore, in the present state of knowledge, it is not yet known if these hormones exert their effects directly by hormonal action, by enzyme modification, or by homeostatic transformations.

In order to fully grasp the tissue modifications under the influence of gestation, it is necessary to recall very briefly the embryology of the female genital tract. This latter develops from Müller's ducts, which in turn is simply the result of an invagination into the genital crest. This zone of mesoblastic origin is closely related to the mesonephros, and it is known that the mesoblast has the possibility of becoming connective tissue, muscular tissue, or even epithelial tissue, as proved by the components of the uterus and uterine tumors.

Thus, the myometrium and the endometrium are composed, respectively, of smooth muscle fibers, and of a tissue made up of glandular tubes of epithelial appearance and of endometrial stroma. This endometrium conserves its embryonic particularities and regenerates within 14 days; its endometrial stroma keeps its mesoblastic potentialities, and in its proliferation is capable of forming epithelial tissues, the glandular tubes.

Moreover, the so-called carcino-sarcomas of the uterus show a tumoral evolution bearing simultaneously on the two constituents of the endometrium, giving rise to a leiomyosarcoma, with which is intimately mingled an adenocarcinoma. Finally, the so-called mixed uterine tumors are sarcomas, whose myxoid, chondroid, osteogenic and muscular elements recall the various potentialities of the original mesoblast.

These diverse possibilities are not an exclusive trait of the genital tract. All of the pelvic connective tissue, in fact, and even the peritoneal tissue, may retain this faculty of metamorphosis. For proof of this, we have only to recall the unexpected localizations of certain umbilical ectopic implantations (1) or abdominal implantations (2, 3, 4), or the occurrence of endometriomas in very diversified zones.

With these facts in mind, we are in a better position to understand the modifications that take place during pregnancy.

A. CONNECTIVE TISSUE MODIFICATIONS

1. The cells of the connective tissue

The cells of the endometrial stroma, and also often those of the entire genital tract, become decidual. They round out and become enlarged, their cytoplasm becomes eosinophilic, with fine vacuoles, and their individual nuclei, slightly displaced toward the periphery, are regular, hypochromatic, and contain fine reticulin.

This decidual reaction commences around an ovum immediately after implantation, then rapidly becomes generalized throughout the entire endometrium, and in 40% of the cases extends into the entire pelvic zone: ovaries, tubes, uterine cervix and the lymphatic ganglia. The cells begin in groups, most often in small masses around the lymphatics, forming nodules of a miliary aspect. The relationship between these transformations and endometriosis will be mentioned later.

2. Ground substance

It is unnecessary to take up the whole discussion of the constitution of the ground substance at this time. A review of the principal studies on this subject may be found in the well-known works of biochemists (5, 6, 7), as well as in those of the histologists and pathologists (8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19).

But the problem of the ground substance as related to pregnancy is an extremely difficult one to study:

a. During pregnancy there is noted an intercellular and interfibrillar edema with an increase in hyaluronic acid. This edema is pelvic but in certain cases may extend throughout the entire organism, accompanied by retarded dermic absorption.

b. At the end of pregnancy, under the action of relaxin (20), the connective tissue of the pelvic belt "depolymerizes" and stains more intensely with PAS, whereas the Evans blue localizes in this region (21, 22).

It is difficult to know whether the modifications are due exclusively to the ovarian hormones (estrogens-progesterone), exclusively to the relaxin or their associated action, or perhaps to their potentialization with the adrenal hormones (cortisone, aldosterone, etc.) and those of the thyroid. Moreover, it is likely that these hormones act through the medium of the "mast cells" which become more numerous during pregnancy. In particular, certain endometrial cells studied (23, 24) are very abundant in pregnancy, as well as the "pale cells" (25) which certain authors consider to be producers of serotonin.

The hormonal actions (maternal, placental or fetal) related to gestation determine marked modifications at the level of the connective tissue and more particularly at that of the pelvic nuclei, namely:

a. decidual transformations on certain particularly sensitive cells.

b. changes in the ground substance, whose constitution varies.

Here, the hyaluronic acid appears in large quantity, and there is a depolymerization of the other glycoprotein constituents, manifested by a more distinct localization of the Evans blue, as well as a retarded power of diffusion. Various enzymes are activated by the different hormones, which are secreted in much greater quantities.

B. EPITHELIAL MODIFICATIONS

The epithelial modifications during pregnancy have long been known, especially the thickening of the vaginal and cervical malpighian mucosa, whose exfoliation Papanicolaou described in 1925 in the form of navicular cells or "oyster-form" cells. But the problem becomes more important in the light of the frequency of abnormal pictures, in particular, "carcinoma in situ-like" formation, determined by pregnancy.

1. Modifications of the Squamous Epithelium

a. In addition to the hyperplasia of the epithelium, there is noted a hyperactivity of the basal layers, characterized by the presence of slightly-differentiated cells encroaching on the zone of the spindle-cells, and by numerous mitotic figures. Three degrees of hyperactivity are to be distinguished.

Slight hyperactivity. The number of layers of basal cells does not exceed five to seven, and their polarity is conserved.

Moderate hyperactivity. To the increase in the number of basal layers is added the persistence of nuclei of active basal type, sometimes mitotic, most often barely undulated in the superior layers. Their exfoliation gives dyskaryotic cellular patterns.

Severe Hyperactivity. This, characterized by an epithelium in which the transformations no longer follow the line of normal evolution, is out of the scope of this paper. Moreover, in a preceding paragraph the question of dysplasias and of carcinoma in situ during pregnancy has already been discussed.

Be this as it may, basal hyperactivity is found, at one or another of the above-indicated degrees, in 4% (26), in 5% (27), in 6% (28), in 6.8% (29), in 14% (30), in 30% (31), and in 40% (32) of the bases. However, these marked divergencies disappear if slight hyperactivity of the basal layers is considered as an anomaly.

b. Squamous metaplasia and pregnancy. It is, nevertheless, useful to note the role of gestation in the hyperactivity of undifferentiated cells, in particular, of the reserve cells or replacement cells.

Very early Carl Ruge, and later Robert Meyer, described these elements which are situated beneath the basal cells of the squamous epithelium and under those of the columnar epithelium. These elements are cells which have conserved their mesodermal embryonic potential, and whose role in the phenomena of the repair of erosions, of ectopiae and ectropions, or even of the whole length of the endocervical canal, is evident.

Pregnancy determines a hyperactivity of these reserve cells, resulting, by metaplasia, in the epidermization of the lesions, so that dystrophies appear which never occur in a normal endocervix. Certain of these dystrophies present images relating them to true vicious cicatrices.

Furthermore, similar patterns of basal hyperactivity and of metaplasia are sometimes encountered in the fetus and the new-born infant; they are due to impregnation by the maternal hormones and are reversible.

Thus, pregnancy determines an activiation of the elements of the basal layers of the epithelium and of the reserve cells of mesodermal potentiality.

From this fact we might presume that the action of pregnancy could be summed up as an increase in cellular activity. In this case there would seem to be little difference between the action of pregnancy and that of estrogens. However, a curious phenomenon should be noted: during pregnancy, the squamous epithelium never advances completely to maturation; the intermediate cells, although in more numerous layers than normal, never become eosinophilic and karyopyknotic, and sometimes exfoliate in a characteristic form, the "navicular cell."

These cellular aspects testify to:

1. the persistence of one or several factors blocking the proteins of SH-radicals which appear at the time of pre-keratinization and retention of glycogen.

2. the inhibition of nuclear pyknosis.

In the case of the navicular cells, there is one modification which could be interpreted as an effort at transformation: a rolling up or thickening of the cytoplasmic border, displacement and, especially, drying up without pyknosis of the nucleus, which shrinks and becomes longitudinally folded. These facts are important in the study of severe basal hyperactivities. The morphological study of these elements may perhaps be the means of differentiating between true cancer and reversible dystrophies.

C. GLANDULAR EPITHELIAL MODIFICATIONS

These modifications (28, 30, 31, 33, 34) may be summarized as follows:

Glandular and adenomatous hyperplasia of the glands.

Hyperplasia of the epithelium, with multiplication of the epithelial layers.

But here, as in the squamous epithelium, extremely hyperplasic nuclei are found, but without anomalies. Sometimes these nuclei are actually "obese," and undergo slight withering which shrinks and creases them, giving them an abnormal aspect.

ROLE OF THE HORMONES IN THESE EPITHELIAL MODIFICATIONS

Hellman and co-workers believe that the occasional excessive activity of the basal layers and the maximum tendency towards squamous metaplasia at the site of all the ectopions and cervical polyps and on the endocervical mucosa, are due to the excess of folliculin.

However, as a matter of fact, experiments on humans as well as on animals show that this hormone, while it determines a hyperplasia of the basal layers, also brings about complete cellular maturation with keratinization.

It is the simultaneous action of the chorionic hormone and of estrogen in the non-castrated animal, or of progesterone and of estrogen in high doses in the castrated animal, which determines the appearance of a pattern similar to that of the epithelium of pregnancy – hyperplasia of the basal cell layers, modification of the PAS and mucicarmine-positive cells.

These modifications are much more marked at the level of the glandular tubes of the endometrium. In the normally progressing pregnancy there is noted an almost imperceptible transition from the premenstrual state of the non-gravid endometrium to the very early decidual reaction following fertilization of the ovum. The glands of the deciduosis present a saw-tooth aspect and a marked coiling; the epithelium is low, stains easily, and is actively secretory; later on, this epithelium flattens out.

But when the pregnancy is interrupted, or when there exists a hydatiform mole or a chorioepithelioma, there appear marked cellular anomalies (35, 36, 37, 38, 39):

hypertrophy and enormous vacuolization of the cell,
nuclear hyperplasia, with loss of polarity.

Identical patterns are likewise found in the cells of the columnar and secretory epithelium of the endocervix.

Certain variations of these basic phenomena should be noted, namely:

proliferative activity of certain nuclei with mitoses, and pseudo-stratification; or, on the contrary, a tendency of the nucleus to shrink, terminating in a sort of condensation of the chromatin; folding of the nucleus, but without pyknotic character.

The endometrial stroma shows cells having identical traits. The decidual cell loses its characteristic aspect. The nucleus recovers its proliferative activity, and mitoses are noted. All of the intermediate stages are to be found on the postabortum endometrium. The postabortum group of cases is mentioned not with the intention of describing such lesions, but merely in order to call attention to the comparison between the images encountered in the ectocervical epithelium during certain pregnancies, and the architectural and cellular anomalies considered as carcinomas in situ.

Hence, the disturbed hormonal actions may possibly be at the root of the cellular modifications noted during pregnancy, if, as would seem to be proved by the animal experiments mentioned above, the hormonal actions during pregnancy determine:

(a) hyperactivity of the basal layer by action of estrogens,

(b) inhibition of cellular maturation by action of the progesteronal hormones, either ovarian (corpus luteum) or chorial,

(c) accumulation of cytoplasmic glycogen, and inhibition of the SH-radicals necessary for cornification, possibly by persistence of the last-mentioned hormones.

However, even though cytoplasmic maturation does not take place, nevertheless, the nucleus withers and shrinks; it assumes an elongated form, with longitudinal furrows, forming folds in the nuclear membrane (resembling in aspect a half-deflated toy balloon) without, however, being truly pyknotic.

When this phenomenon appears at the level of the cells with nuclear "obesity," as is seen in certain dyskaryoses of pregnancy, some very abnormal pictures may be formed which strongly resemble those of malignant cells.

Hence, one may pose the question as to whether "hormonally imbalanced" pregnancy would not be particularly likely to determine cellular anomalies, or an acceleration of the phenomena of metaplasia on the ectropions, so frequent during pregnancy. In such a case, the abnormal architectural and cellular characters would terminate in anomalies similar to those of a carcinoma in situ. And the return to normal of the hormonal imbalance of pregnancy (provided placental vestiges with chorionic action do not persist long) would bring about the regression of the cellular dysmetabolisms.

Inherent in the pregnant state are elements, probably hormonal, capable of modifying the morphology of the connective tissue cells and the homeostasis and chemical constitution of the ground substance. Certain factors, probably these same hormonal elements, determine a hyperactivity of the epithelial cells, with a florid cellular state persisting for a much longer period than is normally seen in the non-pregnant state. It is probably these modifications of the cellular metabolism which are the cause of certain "carcinoma-like" images. More or less well-known hormonal disturbances whose effects are clearly visible following incomplete abortion or in hydatiform moles, may be considered as responsible for some of these anomalies.

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DISCUSSION

J. H. MÜLLER, Zurich, Switzerland:

I would like to congratulate de Brux for having so concisely presented and discussed so many of the most important aspects of the tissue modifications which occur during pregnancy.

This excellent paper could be considered as a basic framework for further, still more detailed, investigations and discussions. It recalls not only important "classical" (however, partly forgotten) ideas, but points also to many important recent advances in the scientific knowledge of the complex and intricate biological phenomena which arise in the pregnant organism.

More specifically, I find de Brux's view that "hormonally imbalanced pregnancy" is very likely to determine all kinds of cellular anomalies, particularly dysplasias, quite a valuable conception. These particular pregnancy-related dysplasias cannot be appreciated correctly with regard to their prognostic significance until they have been checked and rechecked after the last remnants of the pregnancy have disappeared.

Such critical appreciation of dysplasias, correlated with the pregnant status has been for many years our own policy in the field of diagnosis of extremely early uterine carcinoma. Such careful appreciation is, in fact, readily possible, using all the means available, i. e., colposcopy, multiple biopsies and preferably the serial section of ring-biopsies, repeated curettage, cytology of smears, etc. This is, however, not possible for "pregnancy induced" dysplasias in other parts of the organism, in particular in the breast. This would explain the likelihood (from clinical experience) of a probably significant increase of cancer of the breast after a "hormonally imbalanced pregnancy."

I believe that the whole problem treated in de Brux's paper is a quite important one.

NO CLOSING REMARKS

NORMAL VAGINAL CYTOLOGY DURING PREGNANCY

EMMERICH von HAAM AND THOMAS D. EFSTATION
Columbus and Tiffin, Ohio, U.S.A.

Vaginal smears are used more and more by the practicing physician as an aid in the evaluation of hormonal disorders of the female sex glands and the recognition of functional disorders of the female genital tract (1). During pregnancy smears are obtained which reflect the rising progesterone and estrogen levels and therefore have been called characteristic for this condition (2, 3). Those smears contain the glycogen-rich parabasal cells and the glycogen-rich intermediate cells which have the characteristic navicular form and therefore are classified as navicular cells. Navicular cells from pregnant and non-pregnant women can be distinguished by the more compact grouping, the heavier cell outlines and the larger nuclei. However, this difference is not sufficient to speak of "pregnancy cells" or to utilize the vaginal smears for pregnancy diagnosis (4). Navicular cells can compose up to 80 per cent of the cells in the smear of a pregnant woman and such smears have been considered as characteristic for pregnancy under normal hormonal conditions. In addition to the navicular type of smear, we observed the cytolyzed type, in which an increased number of Döderlein flora produces marked cytolysis of the epithelial cells without eliciting an inflammatory exudate. Infections of the cervix during pregnancy may produce a smear with a decreased number of navicular cells, many leukocytes and histiocytes and an increased number of superficial epithelial cells. The cells also show the usual cytoplasmic changes of inflammation and often a pronounced eosinophilia. Those types of smears are identical with similar ones in non-pregnant women. They are obtained in a much higher degree if the smear is taken from the cervical os or the mucous plug occluding the os, rather than if it is taken from the vaginal aspirate or mid-vaginal scraping. In a relatively small percentage of pregnant women smears are obtained showing a demonstrable lack of progestational effect and a rather pronounced estrogenic effect.

Persistence of this type of smear during the first trimester of pregnancy has been considered as a warning signal of threatened abortion, particularly when serial examinations demonstrate a change from a previously navicular type towards the estrogenic type (4). Investigations of Pierce and Cope (5) have shown that patients with an estrogenic smear and bleeding during the first four months of pregnancy had a higher incidence of abortion, and they concluded that the estrogenic smears in these patients indicated the presence of an abnormal decidua or placenta. In comparing the results of Wied, Artner, and Koller, Smolka and Soost pointed to the amazing similarity of the percentage distribution. He also emphasized that the five types of smears are not restricted to certain periods of pregnancy and that one type can easily change into another.

We have followed the cytological picture as it occurred during the first 20 weeks of pregnancy by studying 1000 smears from 786 pregnant women and would like to report our results in the following table.

TABLE 1

Percentage Distribution of 1000 Vaginal Smears of Pregnant Women during the First Five Months of Pregnancy

Week Gestation	Navicular Cell Type	Cytolysis Type	Inflammatory Type	Large Cell Type with Vesicular Nuclei	Karyopyknotic Cell Type
3	15	0	29	41	15
4	16	0	31	38	15
5	30	0	17	41	12
6	31	8	30	17	14
7	34	7	32	19	8
8	47	5	35	12	1
9	51	8	20	20	1
10	48	9	27	13	0
11	49	6	27	18	0
12	51	15	25	9	0
13	56	12	20	8	4
14	55	19	18	6	2
15	57	23	12	8	0
16	53	28	13	4	2
17	56	20	19	5	0
18	58	20	11	10	1
19	62	21	8	9	0
20	60	22	8	10	0

The smears were prepared in most cases from cervical scrapings after visualization of the cervix, which explains the large number of inflammatory types of smears. Inflammatory processes in the cervix during pregnancy usually lead to a decrease of the typical navicular cells and an increase in the number of parabasal cells and large intermediate cells. In the later periods of gestation the number of inflammatory smears declined markedly. The cytological differentiation between the large cells with vesicular nuclei and those with pyknotic nuclei was sometimes so difficult that a separation of the two types seemed rather arbitrary. Smears classified as the navicular type always contained some large cells with vesicular nuclei, but navicular cells, either singly or in small groups, were the predominant type. Differential counts or determination of the Karyopyknotic Index did not seem to alter appreciably the classification of smears, when performed by an experienced cyto-technologist.

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BERTHOLD B. HOCHSTAEDT

Haifa, Israel

Since hormonal activity is one of the essential factors influencing the behavior of the vaginal epithelium, the "normal cytology" during pregnancy described here is concerned exclusively with the hormonally well-balanced and undisturbed pregnancy. Furthermore, it is concerned only with the cytological pattern in the presence of a healthy vagina, i. e., in the absence of infection (Trichomonas, Monilia, etc.) or other inflammatory processes.

After fecundation and during the first two to three weeks of pregnancy the vaginal smear differs only slightly from that of the postovulatory phase of the menstrual cycle. Gradually, according to the

progressing prevalence of progesterone, the cytological picture becomes more typical. In the subsequent course of normal pregnancy there occur several vaginal smear patterns. The most frequent one is characterized by a distinct predominance of cyanophilic cells, most of them derived from the intermediate layer of the vaginal epithelium. The cells present themselves in groups or in compact clumps. The superficial cells are polygonal, most of them crowded, with folded edges and wrinkled cytoplasm. Among the cells of the intermediate layer there appear two types: the usual intermediate cells and the so-called navicular cells (1). The latter is always cyanophilic, smaller than the superficial, occasionally larger than the usual intermediate cell, with doubled-over edges, and folded, frequently vacuolated cytoplasm.

The appearance of the navicular cell type is not characteristic of pregnancy; it occurs following the action of hormones with progestational activity and can be induced experimentally (2). In this vaginal smear pattern leukocytes are rarely found and red blood cells do not appear. Up to the third month of pregnancy the number of navicular cells may not be large, and the superficial cells appear relatively more abundantly, and occasionally are flat. The percentage of eosinophilic cells is not more than 15% and the percentage of karyopyknotic cells is not more than 20%. From the end of the third month or the beginning of the fourth month folding and crowding of the cells become more accentuated, with the number of navicular cells increasing substantially. The superficial cells decrease in number and dimension, frequently almost completely disappear. The percentage of eosinophilic, karyopyknotic superficial cells becomes less than 10. From then on, if pregnancy progresses normally, the cytological picture of the vaginal epithelium does not change as the patient approaches term. Then the desquamative activity of the vaginal epithelium decreases, and a slight increase in the height of cellular proliferation occurs. The large, polygonal, squamous cells with vesicular nuclei appear now with vague outlines and ill-defined cytoplasm. An increase of leukocytes and of mucus give the smear a "dirty" appearance.

Among our series of normal pregnancies, in about one-tenth of the cases studied, there occurred the so-called cytolytic type of vaginal smear. This cytological picture is evidenced by a dominant abundance of Bacillus vaginalis Döderlein (which appear longer than usual), many free, anisokaryotic nuclei and a lack of leukocytes. There is a definite preponderance of intermediate cells, most of them densely packed. The cells appear ill-defined, with vague outlines and in a major proportion of them the cytoplasm may gradually disappear.

Clinically, colpocytology has no particular value as a diagnostic method of early pregnancy, but it presents a highly valuable procedure in the diagnosis of the hormonal equilibrium in the disturbed pregnancy, as well as a reliable guide (3) in the treatment of threatened and habitual abortion.

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HERBERT E. NIEBURGS
New York, New York, U.S.A.

The hormonal activity characteristic for the gestational period and its effect on the vaginal epithelium is reflected in the cells of vaginal smears. With the onset of pregnancy the cyclic changes of the vaginal epithelium are interrupted. The transition from the secretory to the proliferative phase does not occur. The increased estrogen and progesterone activity in pregnancy produces increased proliferation of the vaginal epithelium and increased desquamation of cells with absence of karyopyknosis in the superficial epithelial layers. In the vaginal smear the number of cells increases accordingly and karyopyknotic cells are few or absent except in abnormal conditions. The cells may be smaller than during the secretory phase with oval or rod-shaped nuclei and cyanophilic cytoplasm. These cells, originally described by Papanicolaou as navicular cells, appear in dense clusters and may decrease in size as pregnancy progresses. The navicular cell appears to be an intermediate cell, and its nucleus may be eccentric. The number of Döderlein bacilli may be markedly increased, and varying degrees of cytolysis may be found, particularly between the fourth and ninth months of pregnancy. There may also be superficial cells present. There is usually a rich amount of glycogen in the intermediate cells. An alteration of cellular morphology may be observed in vaginal infections, hormonal changes associated with pregnancy disorders and prior to term.

J. PAUL PUNDEL
Luxembourg, Luxembourg

In 1925, when Papanicolaou started the publication of his excellent cytological studies on the human female, they first dealt with the vaginal cytology of pregnancy. But later the cytological research of Papanicolaou and other cytologists was concentrated mainly on the cytological detection of cancer, so that hormonal cytology and especially the cytology of pregnancy remained in the background. Only after 1945 did some American cytologists begin to study the vaginal cytology of pregnancy again, but these first

papers, only a few in number, did not stimulate any clinical interest in the practical use of the vaginal smear in the control of pregnancy. The findings described were based upon rather subjective and qualitative criteria and the terminology, especially the term "cornification," was applied in a contradictory manner. In 1949, some European cytologists introduced numerical criteria in the evaluation of vaginal cytology during pregnancy. This simplified the hormonal diagnosis to a considerable degree, and it now could be based upon numerical, objective criteria. From this time on, the vaginal smear has been introduced as a routine test in many obstetrical clinics, and the results increasingly confirm the practical value of the vaginal smear for the hormonal control of pregnancy.

The purpose of this paper is not to present a complete report of all the possible modifications of vaginal cytology during pregnancy, but only to present those characteristic cytological particularities of the vaginal smear which are of practical value for the cytological control of pregnancy. Vaginal cytology in abnormal pregnancies will be discussed in another part of this symposium.

CLASSIFICATION OF THE NORMAL CYTOLOGY OF PREGNANCY

The vaginal smear presents particular variations at the beginning and at the end of pregnancy, while it shows a constant uniform pattern from the third month until 10 to 14 days prior to delivery at term. This permits a classification of vaginal cytology into two main groups and three different phases. As hormonal and histological controls of the ovaries and the placenta have shown, these three phases of vaginal cytology have a biological reason. The first phase mostly reflects the hormonal activity of the corpus luteum, while the second and third phases are related to the hormonal activity of the placenta. These three phases can be classified as follows:

I. Cytology of the first trimester of pregnancy

   A. The corpus luteum phase

II. Cytology of the last two trimesters of pregnancy

   A. Placentar phase before term, from the third month until two weeks before term

   B. Cytology of pregnancy at term

The typical modifications of vaginal cytology during pregnancy appear only in the smears without cytolysis, so that one has to separate, for practical purposes, the cytolytic cytology from the intact cytology.

I. THE NORMAL NON-CYTOLYTIC VAGINAL CYTOLOGY

Material of this study: The conclusions of this paper are based upon the cytological findings in over 3000 pregnancies whose evolutions were clinically normal with the delivery of a living baby at term. The vaginal smears were collected after the insertion of a dry speculum before any vaginal examination, and care was taken that the smears were collected exclusively from one lateral cul-de-sac of the vagina and not from the cervix. A blind collection of the smears by means of a pipette has, in my experience, no value for the hormonal cytodiagnosis during pregnancy. The smears were immediately fixed in the classic solution of alcohol-ether or in isopropyl-alcohol with glacial acetic acid and stained by the hematoxylin-Shorr technique. The findings presented are in almost complete agreement with the descriptions presented by other authors.

A. THE VAGINAL CYTOLOGY OF THE CORPUS LUTEUM PHASE OF NORMAL PREGNANCY

If conception occurs during the menstrual cycle, vaginal cytology presents the typical premenstrual phase as described by Papanicolaou. However, in the few days prior to the expected menstruation, the frequent disappearance of the luteal cell clusters and the rise of the Eosinophilic and Karyopyknotic Indices does not usually occur, so that the typical luteal picture of the smear persists and continues in the absence of menstruation. But this persistence of the luteal picture in the immediate premenstrual phase in cases of normal pregnancy is not uniform, and in some cases (5-10%) the smear shows a regression of the luteal characteristics.

If menstruation has not occurred, in most cases the vaginal smear continues to show the typical luteal picture of the menstrual cycle, or the transitory immediate premenstrual changes as described above are followed by a return to the typical luteal phase. But if this luteal cytology continues for over two weeks, the smear progressively presents a different pattern. The smear is composed exclusively of intermediate and superficial cells which at first are of the menstrual type. The Eosinophilic Index remains under 10, while the Karyopyknotic Index, which in general is not higher than 12, occasionally shows an elevation up to 20 to 30. Progressively, the cells become a little larger and the thick, premenstrual cell clusters become more dense. The nuclei enlarge and become vesicular, with a fine chromatin network. Progressively, the superficial cells disappear, so that the Eosinophilic Index comes down to 5 or less and the Karyopyknotic Index to 10 or less. The individual intermediate cells tend to take the navicular form, but they resemble the usual navicular cells of the menstrual cycle at the beginning and are mostly

isolated. Only at the end of the third month do the navicular cells become predominant and take on the typical "pregnancy cell type" characteristics as described by Papanicolaou. It is exceptional to see typical pregnancy navicular cells before the second week of absence of menstruation.

These modifications of the vaginal smear during the first trimester of pregnancy vary from those of the second phase in that they can present some quantitative and qualitative changes while the cytological picture of the second phase of pregnancy remains relatively constant (unless cytolysis, infection or hormonal troubles occur). In the first trimester, the curling of the intermediate and superficial cells is less important than during the menstrual phase, while folding is the predominant cytoplasmic modification. But in some cases the folding occasionally disappears almost completely and is accompanied by a rise of the Eosinophilic Index to 30 and/or the Karyopyknotic Index to 30-50. During these modifications, the smear loses its luteal picture and resembles the pre- or postovulatory smear of the menstrual cycle. But, and this is very important for the hormonal diagnosis, these modifications, and especially the rises of the Eosinophilic and Karyopyknotic Indices, are only transitory (a few days) in normal pregnancies, and the smear reverts to the usual luteal picture with the Eosinophilic and Karyopyknotic Indices under 10. The highest rise of the Eosinophilic and Karyopyknotic Indices is most frequently seen at the middle of the second or the end of the third month, and in other rarer cases one can see the transitory appearance of parabasal cells at this moment. If these modifications are only transitory for a maximum of four days, they have no prognostic importance in clinical evolution, since they can appear in entirely normal pregnancies. We, among others, tend to believe that these transitory estrogenic peaks or regressive changes at the end of the third month are due to the fact that the corpus luteum lowers its full hormonal activity at this moment, which first affects its progesterone secretion, while the placenta has not yet obtained its full hormonal capacity in order to replace the hormonal function of the ovaries completely. It is also the moment when some patients present an occasional uterine spotting without any serious consequences, and biologically explains the frequence of abortions at this period. In some patients the transitory rise of the Eosinophilic and Karyopyknotic Indices accompanied by a transitory disappearance of the luteal characteristics of the smear is seen at regular intervals of four weeks, so that one could believe that they reflect the persistence of a cyclic activity of the ovaries in the first trimester of pregnancy.

B. THE VAGINAL CYTOLOGY OF THE PLACENTAR PHASE OF PREGNANCY

1. The cytology before term. After the third month of pregnancy, and in some cases even before this, vaginal cytology takes on a uniform pattern. The typical pregnancy navicular cells become the predominant element of the smear. The folding of the cells is less accentuated than before and the curling of the cytoplasm becomes more prominent. The cells are shed in thick clusters and their nuclei take on the typical rod-like form in over 25% of the cells. The Eosinophilic Index remains under 6 while the Karyopyknotic Index can vary between 0 and 20 (diameter less than six micra or evaluation under phase-contrast microscopy). If one considers only those nuclei with a diameter under five micra as Karyopyknotic, its index remains constantly under 10 in the last two trimesters of a normal pregnancy.

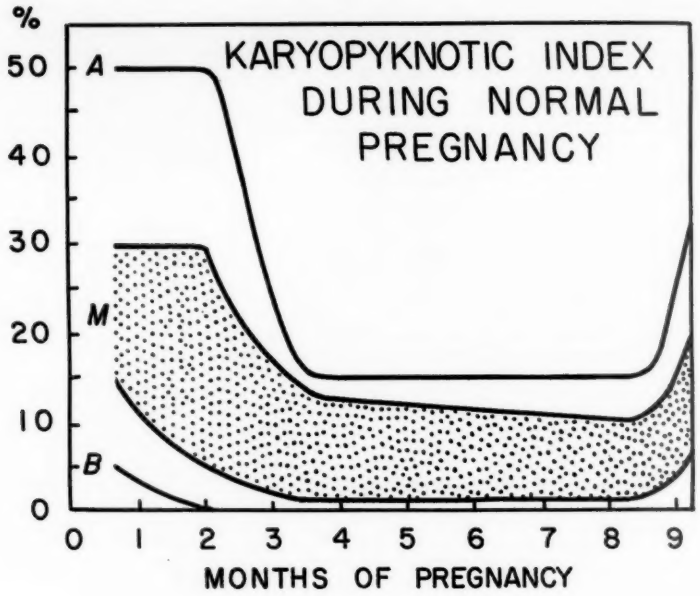
Summarized, the vaginal smear of the last two trimesters of a normal pregnancy is characterized by a constant and uniform pattern of thick clusters of navicular cells, with an Eosinophilic Index under 6 and a Karyopyknotic Index under 20.

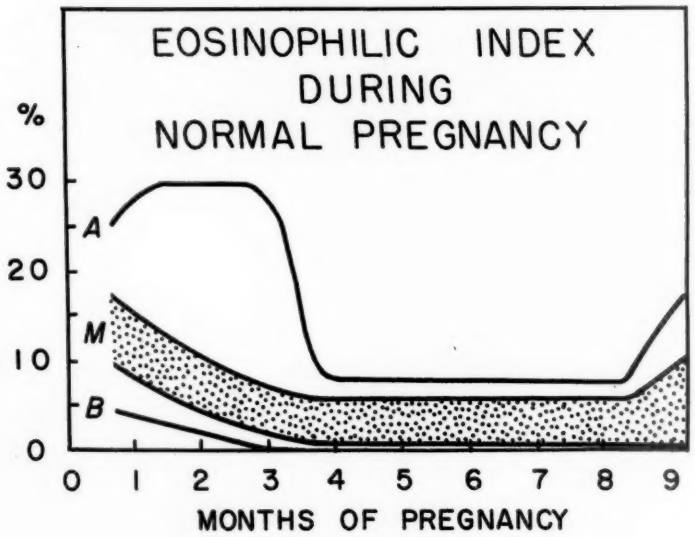
2. The cytology at term. The typical pregnancy smear persists up to the end of the pregnancy, but there appears some particular modification shortly before term which will be mentioned in the next part of this symposium.

II. THE CYTOLYTIC SMEAR

In some cases of pregnancy, and even before the absence of menstruation, the vaginal smear can present a specific pattern. It is composed mostly of free nuclei which are all from the same vesicular type of intermediate cell, surrounded by a massive flora of Bacillus Döderlein. The intact cells are few in number. This is the cytolytic type smear, which can persist during the entire pregnancy. As long as the cytolysis persists, no appreciable modifications are revealed either in the general morphology or in the Eosinophilic and Karyopyknotic Indices, which in general are not higher than 1. As this type of smear presents a uniform pattern during the entire pregnancy by the destruction of the cytoplasm, it is not possible to make particular classifications as is done for the non-cytolytic smears of normal pregnancy.

The question now is if this cytolytic type of smear of pregnancy can be considered as evidence for a normal evolution of the pregnancy; but this point will be discussed in another part of this symposium, and I will be glad to hear the opinions of other cytologists and clinicians.





A = MAXIMAL LIMIT OF TRANSITORY VARIATIONS
M = RANGE OF MEDIUM VARIATIONS
B = LOWER LIMIT

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DISCUSSION

MARCEL GAUDEFROY, Lille, Nord, France:

I quite agree with Pundel. However, some remarks can be added. First, I want to point out that an adequate cytological collection technique is of the greatest importance for valuable studies of hormonal cytology during pregnancy. Pundel has described exactly the procedure of collection of the specimens. I must say the hematoxylin (of Harris or Papamiltiades) -S III Shorr staining, with smears fixed prior to air-drying in alcohol-ether or isopropyl alcohol, alone, gives regular and reproducible results for routine use in hormonal cytology, above all for the hormonal cytology of pregnancy. The Papanicolaou procedure, which is perfect for cancer diagnosis, produces too many shades of colors to make a proper count of cyanophilic and eosinophilic cells. The dried smears, even with special staining, do not give adequate results and must be refused.

In addition, the increases of Karyopyknotic and Eosinophilic Indices do not correspond, in my opinion, to physiological cyclical variations of the ovaries, but are due to a decrease of luteal activity often accompanied by slight bleeding, pelvic pain and uterine contractions which quickly disappear either spontaneously or after administration of progestational substances.

Drs. von Haam and Efstation have established a very interesting picture. However, as Pundel and I have often pointed out, the most sensitive zone of the vagina is the lateral wall and not the cervix. It is, however, remarkable to see that generally the two Indices and the percentages of navicular cells or large cell type with vesicular nuclei correspond with the results published by Pundel and myself.

The papers of Hochstaedt and of Nieburgs entirely agree with my findings and Pundel's work.

MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil:

Pundel stresses the importance of his collection technique of smears for the correct cyto-hormonal evaluation. At this time we are completely convinced that he is right.

In a monograph published in 1953 (1), I described much higher values of the Karyopyknotic and Eosinophilic Indices, in the first trimester of pregnancy, than Pundel and other authors have reported. This was due to a faulty collection technique, since for many years we were accustomed to take up the smears from our gloves immediately after gynecological examination.

However, we do not regret the error because it enabled us to observe certain peculiar changes of the smears which appear at term, shortly before the clinical onset of labor (1, 2, 3). These changes are mainly represented by a sudden shedding of eosinophilic, karyopyknotic superficial cells which, in the smears, appear in a percentage seldom above 30. There are, furthermore, clear regressive changes, besides varying amounts of blood cells. This true peak of the Karyopyknotic Index is of very short duration. As soon as clinical labor begins and progresses, the eosinophilic, karyopyknotic superficial cells tend to disappear from the vaginal smears.

It seems that these cells are shed mainly from the squamous epithelium of the ectocervix at some moment of the "ripening" or effacement of the cervix. Similar changes were observed preceding premature labor and abortion.

We do not know of any endocrinological explanation for this phenomenon, which stands as a critical point in marking the transition to the next biological phase of the vaginal pregnancy cycle: the genital crisis of puerperium.

The classification of the normal cytology of pregnancy proposed by Pundel is most adequate, and it will certainly be widely accepted. We feel, however, that it could be enlarged in order to comprise the whole vaginal "gravido-puerperal" cycle:

I. Cytology of the first trimester of pregnancy

   A. The corpus luteum phase

II. Cytology of the last two trimesters of pregnancy

   A. Placentar phase before term, from the third month until two weeks before term

   B. Cytology of pregnancy at term

   C. Cytology near (or at beginning of) labor

III. Cytology of the postpartum

A. Cytology of the genital crisis, from labor until the return of the normal responsiveness of the vaginal epithelium to estrogens

B. Cytology of the genital recovery, from the return of the normal vaginal response to estrogens until the appearance of a normal estrogenic pattern

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RAIMUND KRIMMENAU, Dresden, Germany:

Our experiences are based upon studies of 1500 normal pregnant women who have had vaginal smears taken.

Since there are considerable difficulties in the routine insertion of a speculum during late pregnancy, the taking of the smear with a cotton swab without unfolding the vagina has proven a valuable method.

Three stages of pregnancy can cytologically be distinguished:

(1) The cytological pattern of the first trimester closely resembles that of the premenstrual phase of the menstrual cycle.

(2) Until approximately 14 days prior to term we encountered the uniform picture with the characteristic navicular cells of pregnancy, lying in dense clusters and displaying some cytolysis.

(3) The third stage again becomes uncharacteristic, i.e., the typical criteria increasingly disappear. Superpositions of (1) and (2) have frequently been observed. In most cases inflammations, especially with Trichomonas infestations, cause the uncharacteristic pattern.

Typical navicular cell patterns have also sometimes been seen in secondary amenorrhea. Thus, we can not give a clear-cut definition of a typical cytological pattern of pregnancy.

JOSÉ MARIA MEZZADRA and GUILLERMO TERZANO, Buenos Aires, Argentina

The definite effect of pregnancy upon the vaginal epithelium can be revealed by vaginal smears as pointed out in detail by the authors: the premenstrual type, the cytologic type and the navicular type.

The changes observed in smears are an indication of the intense and well-balanced estrogen-progesterone activity.

But vaginal smears, though characteristic, are not quite a dependable test for diagnosing pregnancy. Nevertheless, they are a remarkably useful tool in diagnosing pregnancy disorders, if the changes in the Eosinophilic and Karyopyknotic Indices are closely followed.

For hormonal evaluations, vaginal aspirations should be recommended as a more reliable procedure than cervical scrapings.

When infection, irritation, etc., are present, they should always be considered as a possible cause of error.

HANS MUTH, Münster in Westfalen, Germany:

According to our experience I agree with the Main Speakers that functional cytology is not appropriate for the diagnosis of pregnancy. Of the various types of cytological patterns found in pregnancy, we found the navicular or progestational type in only 64% out of a total of 620 normal pregnancies. Thus cytology does not allow the safe diagnosis of pregnancy in a given case. Moreover, the picture is often exogenously influenced by inflammatory changes, e.g., Trichomonas or Döderlein bacilli. However, cytodiagnosis is a valuable tool in following a pregnancy, especially those pregnancies with abortive tendencies, as observed by Pierce and Cope. The exact recording of the Karyopyknotic and Eosinophilic Indices as done by Pundel seems particularly indicated in these cases.

CLOSING REMARKS

EMMERICH von HAAM:

All the discussants seem to agree that vaginal smears are not suitable for the diagnosis of pregnancy per se, but can be used to advantage for the recognition of abortive tendencies. The peak of the Karyopyknotic Index described by Kamnitzer shortly before the onset of labor has also been observed by us, but only occasionally.

BERTHOLD HOCHSTAEDT:

According to my experience I quite agree with Gaudefroy and assume that the increase in karyopyknosis and eosinophilia in the cytological picture of the vaginal epithelium during pregnancy mirrors a depression of luteal activity.

It is gratifying to note that the eminent discussants agree entirely with my findings, as well as with my conclusion as to the value of the cytohormonal diagnosis in disturbed pregnancy.

Despite the mentioned limitations cytology is, for the time being, still the most valuable method for early detection of hormonal disturbances in pregnancy - evidenced by the most important qualities of a diagnostic procedure: reliability and quickness.

HERBERT E. NIEBURGS:

Regarding Gaudefroy's discussion, I would like to suggest that if dried smears are adequately rehydrated and fixed before staining in the laboratory, the results are comparable to those specimens fixed immediately.

We have found Dr. Papanicolaou's staining procedure quite satisfactory for the hormonal evaluation during pregnancy. This, however, may be due to the fact that I prefer to interpret the cells according to their morphology rather than staining reaction unless specific cytochemical methods are used.

I am in full agreement with Mezzadra and Terzano that cervical scrapings are not suitable for hormonal evaluation. However, the alternative of vaginal aspirations suggested is not as satisfactory as smears taken from the mid-vagina.

J. PAUL PUNDEL:

The study of the main papers and discussions shows that there exist no important discrepancies between the findings of the different speakers. I think that this is an important first conclusion.

As the other papers of this symposium on the vaginal cytology during pregnancy will show some minor differences which, in my opinion, are mostly the result of different cytological techniques, I would, therefore, repeat another important conclusion which was pointed out by several discussants: A correct cytological technique is of the greatest importance in order to obtain valuable studies of the vaginal smear during pregnancy. For general use, only specimens collected from one lateral vaginal vault should be accepted. This requires the use of a speculum. Vaginal examinations, douches, intercourse or local treatment should be strictly avoided for at least 24 hours before the collection of the smears. Specimens collected from other parts of the vagina or the cervix do not give the same results and therefore should be used only for special studies or for research work. I agree with Gaudefroy that the hematoxylin-Shorr technique gives the best results for the study of the Eosinophilic Index, while the usual Papanicolaou technique can give particularly high variations of the Eosinophilic Index during pregnancy.

Some discussants have extended their remarks to the cytological diagnosis of pregnancy and to the particular modifications of the vaginal smear before the onset of labor. I think that it would be preferable to discuss these points after the main papers dealing with these questions.

INCIDENCE OF CYTOLYSIS IN VAGINAL SMEARS DURING PREGNANCY

MARCEL GAUDEFROY
Lille, Nord, France

In the use of vaginal smears as a diagnostic and prognostic method of the hormonal disorders of pregnancy, we frequently encounter cytolysis. It is possible to appreciate the incidence of cytolysis, which is in about 50 to 60% of the total smears during pregnancy, and in about 20 to 30% of the smears cytolysis is so marked (almost only free nuclei) that it precludes hormonal diagnosis.

To classify the cytolytic smears I use:

- \- smears without cytolysis
- ± cytolysis less than 15% free nuclei
- \+ from 15 to 30% free nuclei
- ++ from 30 to 45% free nuclei
- +++ over 45% free nuclei

Following up a continuous series of 106 pregnancies, with previous spontaneous abortion or threatened miscarriage, I observed very marked cytolysis in 18 cases, on which 26 hormonal studies (pregandiol, phenolsteroids and 17-ketosteroids) were made, with the hope of determining the hormonal significance of the highly cytolytic smears. Four normal hormonal excretion values were found in two pregnancies, and 22 abnormal values were found in 16 pregnancies (decreased excretion of pregnandiol and phenolsteroids, and normal or subnormal 17-ketosteroids). It was noted that in two cases (with cytolytic smears and abnormal excretion values), cytolysis disappeared at the same time the excretion values became normal.

It may be stated that the strongly cytolytic smear indicates inadequate balance of hormones if all non-hormonal causes of disorders had been treated prior to pregnancy in this series. On the other hand, the cytolytic smears of pregnancy cannot be considered as normal, from the point of view of gestative endocrine balance.

In my opinion, it is probably the insufficient level of the hormonal secretions with particularly a weakness of steroids which is responsible for the insufficient proliferation of the superficial vaginal epithelium, and the lysis of the intermediate cells by Bacillus Döderlein.

However, if one considers the final outcome of these pregnancies (all received estrogenic treatment: diethylstilbestrol according to Smith's scheme), one finds one missed abortion and 17 living children (four premature and 13 at term). Evidently, it is possible to say that the prognosis of those pregnancies with highly cytolytic smears, without being quite normal, still is good with estrogenic treatment.

EMMERICH von HAAM and ROBERT SCHWALLENBERG
Columbus and Tiffin, Ohio, U.S.A.

Cytolysis can be defined according to Pundel (1) as "destruction of the cytoplasm associated with a marked development of Döderlein bacilli." The criteria for a cytolytic smear are the presence of only one type of nuclei (intermediate cells), pure or predominant growth of Döderlein bacilli and marked

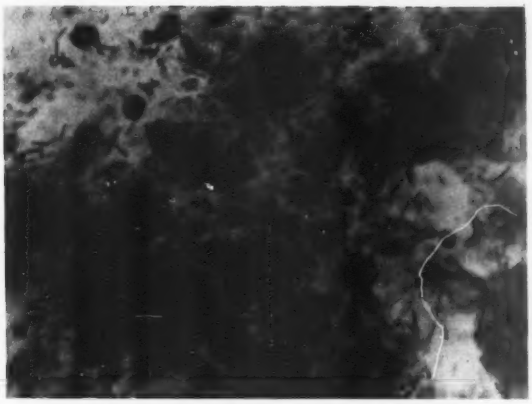

Fig. 1. Naked nuclei, cellular detritus and Döderlein flora typical of cytolytic type of smear. X900

acidosis of the vaginal content (Fig. 1). Its appearance during pregnancy has been repeatedly emphasized, and the cytolytic type of smears represents about 15 per cent of all pregnancy smears in the series of Wied and Christiansen (2) and Artner and Koller (3).

We have studied the occurrence of cytolysis in our pregnancy smears and wish to make the following observations: The cytolytic effect appeared as a rule at a later time during pregnancy than the typical navicular cell type of smear and with few exceptions was not observed before the sixth week of gestation, when it appeared in eight per cent of our material. From then on the occurrence of cytolysis increased rapidly, and after the third month of gestation the cytolytic type of smear was present in 12 to 28 per cent of our pregnancy smears studied at weekly intervals. Cytolysis was more evident in smears obtained by the aspiration method than in those obtained from cervical scrapings, although in the latter all stages of cytolysis involving entire clumps of cells could be noted. During the beginning of cytolysis the eosinophilia of the cytolyzed cells was quite a feature regardless of cell type. In smears with complete cytolysis, however, a certain degree of cyanophilia could again be noted. Women with cytolytic smears did not suffer from an increased discharge, and the pregnancy seemed to proceed in a normal way. Quite often the phenomenon of cytolysis disappeared spontaneously only to reappear at a later stage of pregnancy. It is our impression that it is compatible with normal pregnancy and that no effort must be made to change it into the cellular type of smear by the use of antibiotics.

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J. PAUL PUNDEL

Luxembourg, Luxembourg

Definition: Cytolysis appears very frequently in vaginal smears during pregnancy, varying from beginning cytolysis in a few cells to complete destruction of the cytoplasm of nearly all cells. For practical purposes I consider as cytolytic only those smears which contain over 50 per cent free nuclei and in which the remaining more or less intact cells are not sufficient in number to permit a precise hormonal evaluation based upon the usual morphological and numerical criteria. The basic criteria for cytolysis are: nuclei of the same type and pure or predominant flora of Bacillus Döderlein. These criteria differentiate "cytolysis" from "autolysis."

As it has been demonstrated (6, 7, 9), cytolysis of vaginal cells can appear under different hormonal circumstances, e. g., during amenorrheas, the normal menstrual cycle, pregnancy, after the climacterium or castration and after the administration of estrogens, as well as progesterone and androgens. As the cytological picture of the cytolytic smear is identical in all these different conditions, it will not be possible to make any definite hormonal diagnosis in the presence of vaginal cytolysis.

INCIDENCE OF THE CYTOLYTIC SMEAR DURING PREGNANCY

In my material of over 3,800 pregnancies studied by vaginal smears, the cytolytic type has been found in nearly 6% of all cases in the first trimester of pregnancy and in nearly 15% of all cases in the

second and third trimester. The real incidence of cytolysis would be probably higher if one could take regular smears every week during the entire course of the pregnancy, since the cytolysis is not always a constant phenomenon. In some women the cytolytic smear exists from the beginning to the end of the pregnancy and can be the persistent cytolysis which already existed during the last menstrual cycle. In the majority of cases cytolysis appears in a more appreciable degree only after the third month of pregnancy, because the vaginal epithelium at this time begins to present the optimal biological conditions for massive development of Bacillus Döderlein. However, cytolysis may disappear again under hormonal disturbances or after infections of the vagina and can reappear after treatment of the hormonal dysfunction or the infection.

I have the impression that the cytolytic smear is more frequent (over 30 per cent) in pregnancies complicated by diabetes mellitus, but the diabetes cases in my material are not sufficiently frequent to permit statistical conclusions.

In a series of 400 pregnancies with cytolytic smears during the last trimester of pregnancy, the babies were male in 184 cases, female in 214, and twins of different sex in 2 cases. A typical cytolytic smear with nearly 100 per cent free nuclei was constantly found from the fifth to the eighth month in a patient who was delivered of male triplets.

SIGNIFICANCE OF THE CYTOLYTIC SMEAR DURING PREGNANCY

In a previous paper, I have considered the cytolytic smear as an evidence of a normal pregnancy (6). In another part of this symposium it will be discussed whether this statement can be accepted as correct or not. Some authors (3, 4) believed that the cytolytic smear could have a prognostic significance for the prenatal diagnosis of the sex of the baby. In my material the statistical difference of the sex rates in pregnancies with cytolytic smears is not significant, so that I would conclude with others (7, 2, 5), that the cytolytic smear can not be used as a prenatal test for the sex of the baby.

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DISCUSSION

MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil:

Pundel raises the question if and in what cases should cytolytic smears be regarded as a normal phenomenon in pregnancy.

We were only slightly interested in the study of cytolytic smears until some years ago when we noticed that several patients in the first trimester of pregnancy with this smear type, containing numerous cornified cells, developed symptoms of threatened abortion or actually aborted. This gave us the impression that these findings might indicate a doubtful prognosis of the pregnancy in cases with cytolytic than in cases with non-cytolytic vaginal smears.

In 1952 when we prepared daily vaginal smears from a group of apparently normal pregnant women, we made the following observation:

In the case of a 38-week pregnant woman, from whom we had collected normal vaginal smears during the previous two weeks, there suddenly appeared marked cytolysis. On the same day the fetal heart tones became inaudible. The cytolysis remained unaltered for a few days, and then the smears became "dirty," exhibiting mixed bacterial flora. The vaginal smear showed autolysis and in the subsequent days parabasal and peculiarly rounded intermediate, sometimes chromophobic cells.

The patient was eventually delivered of a macerated fetus. The cause of the intra-uterine fetal death remained obscure.

Later we observed three other cases which exhibited a similar course during the second half of pregnancy.

CAMILLE LICHTFUS, Athus, Belgium:

We have studied 5,000 smears from over 700 patients at the end of pregnancy and found that cytolysis decreases and is never present in cases of true postmaturity. Our results are identical with

Pundel's findings. In smears taken before term we find cytolysis in 22% and in smears at term, cytolysis in only 5%.

We think that it is impossible to give a prognostic value for a possible postmaturity by the evaluation of cytolysis. We believe that it is impossible to determine the term of pregnancy when cytolysis is present.

LUIS MONTALVO-RUIZ, Madrid, Spain:

To Dr. Pundel: I agree with the definition of cytolysis, and I agree that one can frequently see the same pattern in nonpregnant women. We frequently see it in amenorrheas, oligomenorrheas and, in general, in estrogen deficiencies.

We see cytolysis in about 40% of all pregnancies: about 15% are in the first trimester of pregnancy and 25-30% in the second and third trimester.

Without being specific, I think that cytolysis during pregnancy is the response of the vaginal epithelium to a normal pregnancy. When the estrogen-progesterone equilibrium is interrupted as in the threatened abortion when estrogen predominates, and when there are high Eosinophilic and Karyopyknotic Indices present, one can rarely see a cytolytic smear; at least we have not seen it in our series of 100 cases of threatened abortion. If this hormonal imbalance becomes greater and the abortive tendencies are clinically manifested, this would agree with the clinical observation that the hyperestrogenic syndrome rarely goes together with a cytolytic smear type and that many pregnant women arrive at the end of their pregnancy with cytolytic smears and without exhibiting any complications. We also have seen cytolytic smears in some prolonged pregnancies. We agree with Pundel's definitions of cytolysis during pregnancy for prenatal determination of sex.

To Dr. Gaudefroy: We do not completely agree with Gaudefroy, who appears to give the cytolytic smear a pathological meaning during pregnancy because he thinks estrogen therapy should be given. We have seen many pregnant women with cytolytic smears carry their pregnancy to term without estrogen therapy, without any complications.

In Gaudefroy's opinion, hormonal deficiency is responsible for proliferative deficiency of the vaginal epithelium, as well of for lysis of intermediate cells by Döderlein bacillus. We completely disagree with this opinion. We think that during pregnancy there is a considerable proliferation and shedding of the intermediate layer. This shedding occurs so rapidly that the cells do not mature, and therefore we do not find superficial cells. Intermediate cells are full of glycogen providing a good growth condition for Döderlein bacillus, and the conditions that best produce cytolysis exist together.

To Drs. von Haam and Schwallenberg: a) We see cytolysis either before or after navicular type smears in the same pregnancy. They are not related to each other chronologically. We often see mixed navicular-cytolytic smears.

b) Obviously, one sees fewer cytolytic smears before the sixth week of pregnancy, even though we have seen cytolytic smears in the fourteenth and fifteenth days of pregnancy. It may be that this cytolysis already existed during the menstrual cycle prior to pregnancy as Pundel states in his work.

c) We do not observe eosinophilia in cytolytic smears during pregnancy.

HERBERT E. NIEBURGS, New York, New York, U.S.A.:

Cytolysis in vaginal smears is not a rare occurrence in a variety of conditions. It is, therefore, well understood by most investigators and presents at this stage only a few points of disagreement. There does not appear to be any controversy as to the uniformity in nuclear size of cells which have undergone cytolysis and the presence of Döderlein bacilli, although a slight degree of variation may often be noted. The origin of these cells in my opinion is questionable. If, as suggested, they derive from the intermediate layer, then it is most likely the upper intermediate epithelial layer, although often the size of the stripped nuclei may be indicative of the lower superficial layer as the source of origin. Pundel's suggestion of an "Index of Cytolysis" of at least 50% free nuclei as a basis for the term cytolysis is very useful, although Gaudefroy's classification may well be utilized for investigative purposes.

There seems to be some difference of opinion as to the incidence of cytolysis during the individual trimesters of pregnancy. If a cytolytic smear during pregnancy is contrary to Gaudefroy's statement, accepted as normal, the rate of incidence assumes lesser significance. The findings of von Haam and Schwallenberg, regarding the difference of cytolysis between aspiration and cervical scraping specimens, is in agreement with my own observations. Often cytolysis is present extensively in the vaginal smear and not at all in the cervical smear. It would be of interest to have further clarification regarding the statement of eosinophilia and cyanophilia in cytolyzed cells.

CLOSING REMARKS

MARCEL GAUDEFROY:

I am in general agreement with the discussants. However, concerning the remarks of Montalvo-Ruiz and of Nieburgs, I am afraid that I have not made myself clearly understood. In my opinion the cytolytic smears of pregnancy often correspond to adequate hormonal balance, but not always. I shall explain myself. Some authors have written that cytolytic smears indicate normal pregnancy from the hormonal point of view and are prognostic. It has occurred, in cases of strongly cytolytic smears, uninterpretable because of cytolysis, that I have used hormonal dosages of phenolsteroids and pregnandiol and found these hormonal eliminations decreased. I wonder at these findings. In many other comparisons of cytolytic smears and hormonal dosages, in women who had previously had spontaneous abortions, I observed that the hormonal values were below the normal level, and sometimes very low. At first, without treatment, spontaneous abortions again occurred. Later, with estrogen treatment, the pregnancies almost always went to term. Consequently, I recommend that one does not consider the cytolytic smears of pregnancy as always being normal; they correspond, in many cases of habitual abortion, to a decreased level of hormonal balance. However, I have never said that the smears of pregnancy with marked cytolysis were, in all cases, abnormal: I only intended to warn that they were not always normal and that pregnant women with cytolytic smears should be watched carefully, if they previously have had spontaneous abortion.

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J. PAUL PUNDEL:

The main papers and the discussions have shown a rather complete agreement concerning the incidence of the cytolytic smear during pregnancy. However, there remain some minor problems:

(1) Is the cytolytic smear evidence of a normal hormonal balance during pregnancy? As this question will be answered in another part of this symposium ("Normal Vaginal Cytology During Pregnancy") I will discuss only some points which need a better definition. I think that the discussion should consider only the cytolytic smear which shows normal Eosinophilic and Karyopyknotic Indices. With this definition the only difference between the two types of vaginal smears during normal pregnancy is the presence or absence of cytolysis. The problem to be discussed later is the following: Since we know that cytolysis can destroy the superficial cells or block the maturation to superficial eosinophilic cells, therefore, can the cytolytic smear with normal Eosinophilic and Karyopyknotic Indices be considered as a normal smear or is it possible that at least some of these cases would show abnormal smears in the absence of cytolysis?

After these restrictions it will appear that I automatically consider (and I think most other authors, like Gaudefroy, will do the same) every cytolytic smear as being abnormal if it shows abnormally high Eosinophilic and Karyopyknotic Indices. For this reason I think that there remains no disagreement between my own findings and those presented by Kamnitzer. I often have seen similar cases, but I always have considered such smears as being abnormal, since the most important criterion for the diagnosis of the hormonal balance will remain the Eosinophilic and Karyopyknotic Indices and not the presence or absence of cytolysis.

The finding of cytolytic smears in the first days and weeks after the intra-uterine death of the fetus (after the 16th week of pregnancy) is not exceptional, one can even find more or less normal non-cytolytic smears in such cases. I think that in these cases the death of the fetus is independent of and prior to placental changes as shown by many histological and biological studies, while in cases of death or impending death of the fetus secondary to regressive placental changes, the smears will show very early corresponding changes.

(2) Is there a difference between the degree of cytolysis and the side from which the smears have been collected? I think that this question can be answered affirmatively as shown by von Haam, Schwallenberg, Nieburgs, and myself. The cervical epithelium is less sensitive to the cytolyzing effect of the Döderlein bacilli than the vaginal cells. This is another argument for indicating for each specimen the site of collection.

(3) The eosinophilia and cyanophilia in cytolyzed cells. If the cytolysis is complete, it is impossible to discover from such smears if the cytoplasm of these smears, before the start of the cytolysis, has been eosinophilic or cyanophilic. But like Wied, Christiansen and Gaudefroy I have never seen partially cytolyzed cells with remnants of eosinophilic cytoplasm, or cytolysis of superficial cells with naked pyknotic nuclei remaining. I would conclude, therefore, that cytolysis (a term which I understand to mean exclusively the destruction of the cytoplasm associated with a vaginal flora composed mostly of Döderlein bacillus) can occur only in cyanophilic intermediate cells and never in superficial cells with pyknotic nuclei. This conclusion is particularly correct for the hematoxylin-Shorr technique, while with the Papanicolaou technique, if not correctly applied, there can be exceptions in the way that one observes an eosinophilic cytoplasm in partially cytolyzed cells. These exceptions concerning only the staining reaction of the cytoplasm and not the nuclei or the cell type are due, in my opinion, to artifacts caused by technical variations and cannot be accepted as evidence that cytolysis would occur in eosinophilic superficial cells.

VAGINAL FLORA OF PREGNANT WOMEN AS COMPARED WITH THAT OF NON-PREGNANT WOMEN

ARTURO ANGEL ARRIGHI
Buenos Aires, Argentina

There is a difference only in degree between the bacteriology of the vaginal flora of pregnant and non-pregnant women. In the first group we can observe a nearly pure culture of Döderlein bacilli, with its typical aspect of short bacilli or with its filamentous or pseudomycelian aspects, first described by Bourg in 1954.

We also find, in a small group of women, Staphylococcus and Monilia, which possibly are living in a quiet state as non-pathogenic inhabitants of the vagina.

The pH of the vagina (below five), provided by hormonal levels and also by lactosuria, may be the explanation of these findings.

JULES-ANDRÉ BRET, R. LEGROS AND F. J. COUPEZ
Paris, France

In various recent publications we have systematically studied the vaginal flora of pregnant and non-pregnant women.

Two points have come to our attention:

a) The vaginal pathogenic flora can be the cause of early infections of the newborn.
b) In pregnant women, the clinical manifestations are always major.

After extensive tests, we have given up the direct antibiogramm and the standard culture on peptone or gelatine bouillion, as still recommended by Elizabeth Waugh and Anne Pike.

These methods are basically subject to profound errors which come from the culture properties which differ according to the various germs. With these methods certain germs, which are not at all or only a little pathogenic, grow quickly and suppress certain less numerous virulent strains which grow poorly in standard media.

Thus one tests the most frequent, the most apparent strains, which are not necessarily the most virulent ones. That is the reason why we strongly insist upon the technique described here, based upon a culture in a specific medium followed by antibiogramms on isolated germs and not on mixed ones.

We perform the bacteriologic examination of leukorrhea in the following manner:

The specimen is taken in the laboratory itself, the patient having gone without vaginal douches or local treatments for at least three days. The examination starts with a test for Trichomonas by the direct method followed by an inoculation of the different specific media, including inoculations for anaerobic germs: medium of Peizer and Steifen for gonococci, of Kristensen for coli-like and proteus, gelatine of Veillon for anaerobies, of Sabouraud and Nickerson for fungus, of Chapmann for Staphylococci, of Kupfenberg for Trichomonas. The resistency determination of every isolated germ to the different antibiotics completes the study; thus every single patient requires six to ten inoculation plates and resistency determinations.

We have examined: 366 non-pregnant women with leukorrhea
257 pregnant women with leukorrhea

In most cases concerning the non-pregnant women in our gynecological out-patient clinics, a certain selection was already made, because the patients usually came to the clinic with symptoms, often of leukorrhea. In contrast, the bacteriological study of the vaginal flora of the pregnant women was systematically done, every woman having been given a complete examination.

The following table gives the complete results. Realizing that all the women were submitted to the ascribed study, even those who did not show any symptoms, one may conclude that the incidence of vaginal infection is higher in pregnant women than in non-pregnant women.

MICROBIOLOGY	Non-Pregnant Women		Pregnant Women	
	No. of cases	%	No. of cases	%
Trichomonas only	16	4.4	11	4.0
Trichomonas and mixed bacteria	57	15.5	22	8.0
Trichomonas and Staphylococci	22	6.0	11	4.0
Trichomonas and Monilia	4	1.09	9	3.5
mixed bacteria	117	31.9	71	27.6
anaerobes	24	6.5	11	4.0
Staphylococci and mixed bacteria	30	8.1	31	12.0
Staphylococci only	34	9.2	29	11.2
Staphylococci and Monilia	8	2.1	1	0.3
Monilia only	54	14.7	47	18.2
no bacteria visible	0	0.0	14	5.4

The vaginitis caused by Trichomonas, Candida albicans and Staphylococci amounts to 2/3 of all cases of vaginitis in either pregnant or non-pregnant women.

The influence of this infection on the cytological examinations has been studied by Nesbitt and Hellmann, McIlrath and Hellestrand and more recently by Elizabeth Waugh and Anne Pike. Every one of them insisted upon the evidently peculiar role of Trichomonas. In France, this question has been discussed in the symposium on Trichomonas at Reims in May 1957, where Haour and associates reported the results of their studies on dysplasia due to Trichomonas.

This particular infection is not without certain subsequent consequences, especially some cervical modifications as evidenced by combined cytological and colposcopical examinations. Certain germs seem to play a key role, particularly Trichomonas. These modifications are much clearer during pregnancy. This infection can be recognized by the mentioned cytological and colposcopical modifications.

From the cytological standpoint: In our Department of Gynecology and Obstetrics 3,072 pregnant women were examined, and 4,140 smears were taken from June, 1955 to May, 1958.

In 14 cases where the smears were classified as Class III and in several others without classification, the infection seemed to have its cytological counterpart.

With or without anti-inflammatory treatment, this classification was only transitional, and in ten cases we could observe a regression.

In two cases the cytologist diagnosed the smear as Class IV, in all the other cases: Class II, with inflammatory changes. The anti-infectious treatment allowed observation of the regression.

From the colposcopic point of view, one will find the same conditions:

1. All cervices of the pregnant women constitute inflammatory appearances: cervicitis, infected ectropion with a purulent glare, hemorrhagic spots, colpitis with lymphoid islets.

2. Relative frequency of dysplasia, sometimes fugacious and erratic, which was not always malignant upon histological examination. They give, particularly in the case of pregnancy and more so in cases with serious simultaneous infection, suspicious pictures which are hard to interpret. The important role of Trichomonas should be recalled here.

To clearly divide the true dysplasia from the inflammatory changes, one must insist upon:

1. The central role of anti-inflammatory treatment. Only after such a treatment can one draw definite cytological conclusions.

2. The findings on histological sections in case of infection or inflammatory reaction in the site of the stroma with certain condensation of the latter at the site of the basal membrane.

Through the colposcope those pictures may look very alarming: field, ground, base, red zones; but they can disappear very easily.

It is very difficult to say if such an infection is capable of producing such modifications by itself or if it only accentuates and multiplies them.

Anti-inflammatory treatment alone must not permit definite conclusions during pregnancy, in spite of the good "optical" results it may yield. The final therapeutical decision must not be made until several months after delivery, especially in the presence of doubtful histological pictures.

The case which we report here is an illustration of the above statements:

Mrs. CEI, 39 years of age, 5 para, 7 gravida, came for a consultation on April 14, 1958 at 2-1/2 months of her seventh pregnancy. Heavy leukorrhea with cervico-vaginitis.

Colposcopy: Iodine negative zone with ground and field (Fig. 1).

Cytology: NUOVO: Class III, de BRUX: Class V.

Histology: Spinal cell epithelioma with abundant mitoses. Treated with anti-inflammatory measures. On following examinations the progressive normalization could be observed.

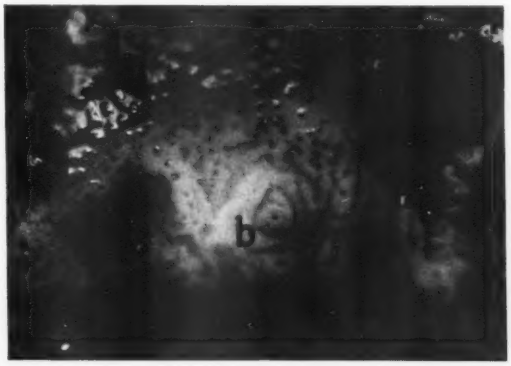

Fig. 1. Mrs. CEI, 4/25/58, magnification 2 x, acetic acid, the anterior lip is seen with a) ground and b) field pattern. The entire anterior lip is iodine negative.
Cytology: Class III and Class V.
Histology: Carcinoma in situ.

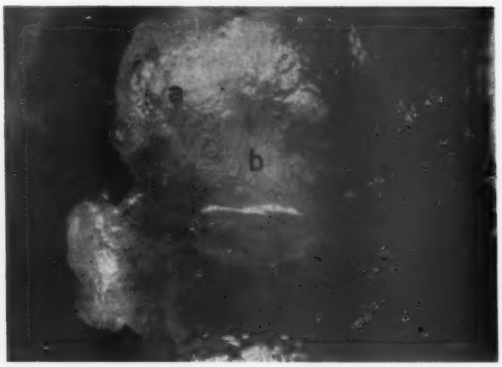

Fig. 2. Mrs. CEI, 4/25/58, magnification 1.1 x, Lugol. Large iodine negative spot, on the surface of which one guesses the ground and field pattern.

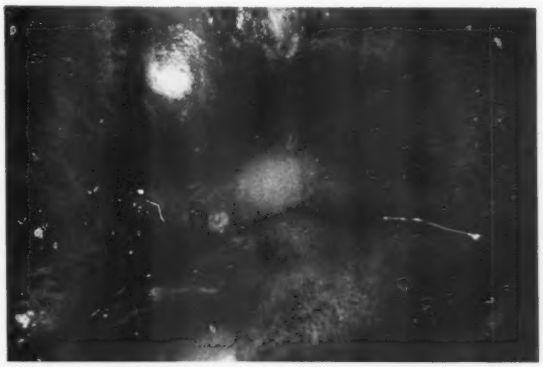

Fig. 3. Mrs. CEI, 7/18/58, magnification 1.1 x, acetic acid. After intensive anti-inflammatory treatment the whole surface of the epithelium seems normal and iodine positive, except the two marked white spots a and b.
Cytology: Class III.
Histology: in a and b, Carcinoma in situ.

Fig. 4. Mrs. CEI, 10/6/58, magnification 1.1 x, Logul. Both cervical lips are epithelized, the lining is not interrupted and iodine positive. The pattern has disappeared except in the two little sites a and b, where still two spots persist, which are iodine negative and are histologically carcinoma in situ.
Cytology: PAPANICOLAOU Class II.

October 6 Colposcopy: Only two small spots persist, the size of needle points.
Cytology: NUOVO: no classification, de BRUX: some ordinary dysplastic element, Class II.
Histology: Still reveals a carcinoma in situ appearance in a section taken from the site of small, remaining iodine negative area.

The anti-inflammatory treatment has therefore well achieved the almost complete disappearance of the colposcopic lesion on the anterior lip. On one site the histological lesion remains.

Will this lesion regress after delivery?

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DISCUSSION

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

The very comprehensive study of Bret and co-workers shows that two-thirds of all cases of vaginitis in either pregnant or non-pregnant women is caused by infection with Trichomonas, Monilia or Staphylococci. In his differentiation of true dysplasia from inflammatory change he stresses the important role of the effect of anti-inflammatory therapy. Our experience agrees with Arrighi that the most important difference between the vaginal flora of pregnant and non-pregnant women is the frequent presence of pure cultures of Döderlein bacilli responsible for the well-known cytolytic effect.

BERTHOLD B. HOCHSTAEDT, Haifa, Israel:

I agree with Bret, Legros and Coupez concerning their conclusion that the incidence of vaginal infection is higher in pregnant women than in non-pregnant women, with the remark that this finding may be related only to the bacteriological culture. From the clinical viewpoint the incidence of symptoms following vaginal infection appears to be significantly lower in pregnant than in non-pregnant women. Reviewing the case histories with bacteriological and cytological findings of 800 women, I found that among non-pregnant women harboring pathogenic Staphylococci, 68% complained of clinical symptoms, whereas among pregnant women harboring pathogenic Staphylococci only 51% had complaints. The incidence of clinical symptoms following Trichomonas infection was lower in pregnant than in non-pregnant women (5.0% as compared to 7.4%).

Thus the occurrence of non-virulent pathogenic bacteria seems to be more frequent during pregnancy than in the non-pregnant woman.

As to the normal vaginal flora: In my experience with cytological examinations of sterility patients who became pregnant after treatment or following artificial insemination, it could be observed that only "difference in degree" developed in most cases, before and during pregnancy, as stated by Arrighi.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

As this symposium deals exclusively with hormonal cytology during pregnancy, I think that the paper of Bret, Legros and Coupez should be discussed in the symposium on cancer diagnosis during pregnancy. I prefer, therefore, to discuss only the problem of the vaginal flora as it appears to the bacteriologist. Together with Dr. Ost I have studied more than 800 specimens of vaginal flora, and the results are nearly the same as shown by Bret and co-workers and Arrighi. Our results can be summarized as follows:

One can identify the same bacteria, fungi and parasites in the vagina of pregnant women as in that of non-pregnant women. The comparison of the vaginal flora of these two groups of patients shows only quantitative differences. For example, Monilia are more frequent in pregnant women, while the coli group is found more frequently in non-pregnant patients. The possible cytological modifications and artifacts resulting from the action of bacteria or parasites, such as Trichomonas, are the same in pregnant as in non-pregnant women, so that it is not necessary to enter into further discussion concerning this problem.

To Arrighi I would reply that the filamentous or pseudofungoid aspects of the Döderlein bacilli have been well-known for over thirty years (1, 2). Bourg has introduced a more precise, descriptive term for this particular form of the Döderlein bacilli which could be easily confused with Monilia in cytological smears if no controls were done by culture.

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CLOSING REMARKS

ARTURO ANGEL ARRIGHI:

I would like to thank Drs. HOCHSTAEDT, PUNDEL and von HAAM for their very interesting comments; this completes the picture of the vaginal flora in pregnancy.

JULES-ANDRÉ BRET, P. LEGROS and FERNAND J. COUPEZ:

We fully agree with the statements of von Haam and Pundel. As to the problem posed by Hochstaedt, we also believe that the changes in the vaginal flora during pregnancy are very significant. Only serial bacteriological examinations have a real value in our eyes. It is true that since the patients most often see their doctor because of a leukorrhea, there is already a selection made, and statistical evaluations are faulty from the very beginning.

Since we are concerned with pregnant women, the only way to obtain valid statistical results would be to screen the entire female population.

In order to judge objectively the modifications of the vaginal flora during pregnancy, it is indispensible to select a group of patients from the same age group. Hence, the premenopausal, and the menopausal women whose vaginal flora is quite different, should be excluded from the very beginning.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

VAGINAL CYTOLOGY AS PROGNOSTIC METHOD IN PREGNANCY DISORDERS

MARCEL GAUDEFROY
Lille, Nord, France

There is a very wide variation in the reported incidence of abortion, but it is generally accepted that the incidence of spontaneous abortion is approximately 15% of all pregnancies. If we consider exclusively the abortions apparently due to endocrine disorders, the incidence is about 6 to 7% of all pregnancies, the other abortions being due to cervical incontinence, uterine malformations, defective ova and other causes, which are without interest in this report on hormonal disorders.

After all possible non-hormonal causes of abortion have been excluded, it is possible to evaluate the following questions by means of cytology: (1) Is threatened abortion present? (2) Is the prognosis of endocrine pregnancy disorders poor or good? (3) Is the administered hormonal treatment successful or not?

As compared with the normal cytological criteria the following criteria of hormonal disturbance are used: disappearance of navicular cells or luteal cells (intermediate, folded cells in clusters), occurrence of cyanophilic and eosinophilic superficial cells in considerable number, Eosinophilic Index increasing over 5-10, and Karyopyknotic Index over 15-25.

(1) CYTODIAGNOSIS OF THREATENED ABORTION. I examined 1450 pregnant women cytologically. In 274 hormonal cytology was abnormal. In 1176 cases (without previous abortions) the cytohormonal pattern was normal. Of these 1176 patients nine aborted (0.77%) and 1167 delivered a living child, premature or mature (99.23%). Pundel (1) found in his 3393 cases exhibiting normal colpocytology, 20 abortions (0.59%) and 3373 normal deliveries, 47 premature and 3326 mature (99.41%).

It is evident statistically that the above percentages of 99.23% and 99.41% respectively are significant as compared with 15% abortions in the overall number of pregnancies. It is almost possible to accurately state that the pregnancy will go to term, if there had been no previous miscarriage and if the vaginal smears were normal.

(2) CYTOPROGNOSIS OF ENDOCRINE DISORDERS IN PREGNANCY. In my practice I had the opportunity to cytologically observe 178 cases of threatened abortions, all of them under estrogen treatment. Table I shows that among the 38 cases showing normal smears, there was one case of blighted ovum and 37 deliveries of living children (3 premature). Among the 140 cases exhibiting abnormal smears, there was one case with abortion (of a normal embryo), 21 cases of blighted ova, 118 deliveries of living children (20 premature, 3 with malformations). Liefooghe applied statistical methods to the above results. The "fiducial limits" (probability P = 0.05) has been calculated. In patients with normal smears in my series the chances of cytoprognostic errors are 2.63% (with limits between 0.06 and 13.1%). In patients with abnormal cytohormonal smears the cytoprognostic chances of error are 15.07% (with limits of error between 9.57 and 21.85%). There is a stastically significant difference between both groups (normal and abnormal cytohormonal smears) with regard to the outcome of pregnancy: $t = 2.16$; $0.02 < P < 0.05$. Vaginal smears have, therefore, real value as a prognostic method in pregnancy disorders.

Table I

		ABORTION		CHILDBIRTH		
					Premature	
		Living Ovum	Blighted Ovum	Normal child	Normal child	Abnormal child
THREATENED ABORTIONS = 178	Normal Cytohormonal Smears 38	0	1	34	3	0
	Abnormal Cytohormonal Smears 140	1	21	95	20	3

For comparative examination with the colpocytological method in 42 cases, the smears and the hormonal excretion values (pregnandiol, G. B. S. 13, phenolsteroids and active folliculin). The results are shown in Table II.

Table II

Cyto-Hormonal Smears	Hormonal Bioassays Normal	Hormonal Bioassays Subnormal	Hormonal Bioassays Abnormal	Total Number of cases	DELIVERY		
					Normal	Premature	Abortion
Normal	12	2	2	16	14	1	1
Subnormal	0	3	0	3	2	1	0
Abnormal	1	0	22	23	16	3	4
TOTAL	13	5	24	42	32	5	5

Of 16 patients with normal cytohormonal pattern 12 showed normal, two subnormal and two abnormal hormonal bioassay values, and 15 of the 16 pregnancies went to term. Of 23 patients with abnormal cytohormonal smears, one showed normal and 22 abnormal hormonal bioassay values. Nineteen of these 23 delivered living babies and four had abortions.

The statistical method (X^2- test) has been applied to these numbers. For the degree of freedom 4, P = 0.001 (P = probability), the X^2 limit for d d l = 18.46. Consequently, there is less than one chance in 1000 for error, which means that the correlation between vaginal smears and urinary hormonal bioassays is statistically very highly significant.

(3) CYTOLOGICAL CONTROL OF HORMONE THERAPY. Finally, vaginal cytology is a good method of prognosis for the efficiency or inefficiency of hormone therapy of pregnancy disorders. Almost at the same time, Bourg and co-workers, and myself, indicated the possibility of making a cytodiagnosis of the death of the ovum during estrogen treatment. Administered estrogens induce no differentiation, no maturation of the vaginal mucosa during normal pregnancy. However, if the ovum is dead, the vaginal epithelium reacts with marked proliferation: navicular or intermediate cells disappear, the number of superficial cells increases, the Eosinophilic and Karyopyknotic Indices increase respectively above 10 and 25. If the causes of diagnostic errors (such as vaginal douches, infections, etc.) are carefully eliminated, the diagnostic accuracy of the cytohormonal prognosis of hormone therapy during pregnancy is very high and clinically useful.

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LUIS MONTALVO-RUIZ
Madrid, Spain

In this paper we are only going to show the results of our personal experience. Accordingly, we shall leave out bibliographical quotations.

Our study is based on 570 pregnant women from the Seguro de Enfermedad de la Maternidad Municipal and from the Second Chair of Obstetrics and Gynecology of Professor Botella. We shall classify them into the following groups:

Group	Classification	
1	Normal pregnant women before term	221
2	Pregnant women at term	78
3	Protracted pregnancy	12
4	Hemorrhage without tumoral damage	105
5	Pregnancy of the first quarter	59
6	Pregnancy of the third quarter	20
7	Infection of the genital canal	55
8	Premature delivery (between 7th & 8th months)	9
9	Placenta praevia	4
10	Dead fetus from 7 to 8 months	3
11	Eclampsia	2
12	Mole	2
	Total	570

This paper is concerned only with groups four through twelve.

RESULTS

Group four includes 105 pregnant women of which two had small hemorrhages during their pregnancy at the time when menstruation was to appear. The vaginal cytology did not show any alterations.

The other 103 pregnant women we shall divide into two divisions:

(a) Hemorrhages during the first three months ..... 72 cases

In 68 cases the cytology was modified, increasing the Eosinophilic Index to between 25 and 50. In seven of these women, in spite of the hormonal treatment, the Eosinophilic Index remained about 50 and all except one of them aborted. With the other 61 women under suitable treatment we could see the Eosinophilic Index decrease below 25 and all of them had a normal delivery.

(b) Hemorrhages during the last six months ..... 31 cases

Thirty women showed an increase of Eosinophilic Index over 6. Four of them reached an Eosinophilic Index of 65. All four aborted. In the other 26 women under treatment the Eosinophilic Index was reduced below 6 and all of them had normal deliveries.

Group 5. Pregnancy of the first quarter: no cytologic anomalies were found.

Group 6. Pregnancy of the third quarter: no alterations in the smears were found.

Group 7. Infection of the genital canal: this group should be studied separately, because infection of the vagina and cervix, especially the ones due to Trichomonas, are processes often precluding hormonal readings prior to specific treatment. In fact, in the 55 cases that we have observed, the navicular cells had disappeared, and there were dyskaryotic cells and an increase in eosinophilia.

Group 8. Premature deliveries: in eight women normal pregnancy cytology, in one case increase in the Eosinophilic and Karyopyknotic Indices over 25 and 40 respectively.

Group 9. Placenta praevia: in two cases normal cytology, in the other two cases cytolytic smears with an Eosinophilic Index over 50.

Group 10. Dead fetus between seven and ten months: in the three cases the cytology was definitely altered with disappearance of the navicular cells and appearance of some parabasal cells of the postpartum type.

Group 11. Eclampsia: no alteration in the smears were found.

Group 12. Mole: in both cases the two indices (E.I. and K.I.) were raised between 30 and 50 respectively.

SUMMARY: The main alterations of the vaginal cytology to establish the prognosis of pregnancy are based on the increase of the Eosinophilic and Karyopyknotic Indices, disappearance of the navicular cells and appearance of the parabasal cells of the postpartum type.

J. PAUL PUNDEL
Luxembourg, Luxembourg

The vaginal smear of pregnancy has been studied for over 30 years, but only during the last decade has it been considered a valuable test for the hormonal control of pregnancy. Before 1950, the evaluation of the vaginal smear for hormonal diagnosis was based upon the general cytological pattern of the smear, depending, therefore, mostly on the subjective judgment of the cytologist. But as long as the vaginal smear was evaluated merely by gross examination of the cell picture, it was not possible to achieve precise hormonal information. The introduction of numerical criteria in the vaginal cytology of pregnancy by Gaudefroy and Van Meenzel in 1950 marked the beginning of a new period. The vaginal smear became an objective test, and all of the following studies, published mostly in European countries, show more and more the practical value of the vaginal smear for the general obstetrical practice.

It is not possible to give precise hormonal evaluations, if one deals only with the general morphology of the cells. For a precise and reproducible cytodiagnosis there are now two objective numerical criteria, the Eosinophilic and Karyopyknotic Indices, which permit critical and objective reports of the cytological findings. In a previous part of this symposium the normal cytology of pregnancy is presented and the limits of the normal vaginal smears for the different phases of the pregnancy are described.

In rather extensive material, I have studied independently and simultaneously with Gaudefroy the vaginal smears of pregnancy and compared the cytological findings with the clinical evolution. All smears have been stained according to the hematoxylin-Shorr method. This study was done in order to examine the prognostic significance of the vaginal smear for the progression of pregnancy and the success of treatments for hormonal disorders.

A. THE PROGNOSTIC SIGNIFICANCE OF THE NORMAL PREGNANCY SMEAR

In 3,393 pregnancies the vaginal smear was normal, within the limits previously described. In 3,326 cases the pregnancy was terminated at term with the birth of a living child (except in nine cases where the infant died during abnormal delivery). In 47 cases, the pregnancy was terminated due to premature delivery. Intra-uterine death of the fetus after the sixth month occurred in 12 cases, and eight patients had a spontaneous abortion. It seems that this successful course of the pregnancy in over 99 percent of the cases, nearly the same as in the material of Gaudefroy, is statistically significant.

Accidents to the child occurred only in an insignificant number of cases, and the follow-up of these cases proved that they were due to factors other than hormonal. The cause of abortion was in all cases (as proved by histological examination) a blighted ovum, and the death of the fetus before delivery, as well as the premature deliveries, were attributed to non-hormonal factors, such as erythroblastosis, toxicosis, uterine and fetal malformation, hydramnions, twins and triplets, uterine tumors and placenta praevia.

In a previous paper (30) the cytolytic smear of pregnancy has been described as evidence of normal hormonal activity. This conclusion has been based only upon the clinical course of the pregnancy. Meanwhile, Gaudefroy (12, 13) studied a series of pregnant women with cytolytic smears with hormonal bioassays and found that some hormonal disorders may also exist in women with cytolytic smears. Gaudefroy included in his series all smears from beginning to complete cytolysis, whereas, I consider as "cytolytic" only smears which contain more than 50% free nuclei, with Eosinophilic and Karyopyknotic Indices each below 1. In a total of 450 cases with cytolytic smears only three abortions were noted. Later it was found that these abortions had been induced by the patient (injections of soap in the uterus). In five cases the fetus died in utero (erythroblastosis, diabetes or fetal malformation). In 19 cases the pregnancy ended with premature delivery from other non-hormonal causes, such as hydramnions, twins or placenta praevia. The findings of Gaudefroy are very interesting, and the recent investigations concerning the etiology and mechanism of cytolysis may explain the existence of cytolysis in cases with even marked hormonal dysfunctions as observed in the non-pregnant woman. One should expect to find in such cases abnormally high Eosinophilic and Karyopyknotic Indices, but if the hormonal dysfunction starts in patients who previously had a cytolytic smear, we now know that persisting cytolysis will inhibit the development of eosinophilic superficial cells. Cytolysis will destroy the cells before they achieve this final stage of epithelial differentiation, which would correspond to the actual hormonal stimulation.

Gaudefroy in his material observed 18 cases, with abnormal hormonal assays in 16 of them, all of which had been treated by estrogens. There occurred only one abortion. It was a missed abortion at the time of the first smear. From this, Gaudefroy concludes that the pregnancy of a patient with a cytolytic smear has a good prognosis with estrogen treatment. Despite the good results in my own material without any hormonal treatment, I think now that the findings of Gaudefroy should be considered with attention, and I would like to make the following correction of my previous statement.

In general, the cytolytic smear during pregnancy indicates a good prognosis even without any treatment, but as cytolysis can conceal abnormal cytological modifications which would appear in a non-

cytolytic smear, it seems to be prudent to inhibit cytolysis with short local antibiotic treatment in order to obtain a non-cytolytic smear, which now would reveal possible hormonal disorders and permit their early treatment.

B. THE PROGNOSTIC SIGNIFICANCE OF ABNORMAL PREGNANCY SMEARS

Nearly all reports published in the last few years conclude that the vaginal smear is an excellent test for the early detection of hormonal dysfunctions. In my series of 3,800 cytologically examined pregnancies the vaginal smear was abnormal in 407 cases. Of these 407 cases 117 showed no clinical evidence of impending abortion at the time of cytological examination. Twenty-eight patients refused prophylactic treatment. Of these 28 only 11 had a successful pregnancy (four deliveries at term and seven premature deliveries). In three cases, the fetus died in utero, and in 14 cases spontaneous abortion occurred between the fifth and the 37th day after the initial vaginal smear. Eighty-nine patients received immediate hormonal treatment, and of these, 60 had a successful delivery (49 deliveries at term and 11 premature deliveries). In eight cases death of the fetus in utero occurred later, and 21 patients had an abortion in spite of the hormonal treatment. If we consider only the cases with abortion, the abnormal vaginal smear in clinically normal patients was followed by spontaneous abortion in 35 cases or 33.4 per cent, despite hormonal treatment in the majority of cases. This difference with the frequency of abortions in patients with normal vaginal smears is statistically valuable and highly significant.

C. THE PROGNOSTIC SIGNIFICANCE OF THE VAGINAL SMEAR IN CLINICALLY-APPARENT, THREATENED ABORTION

If a pregnant woman presents herself with vaginal bleeding and/or pelvic pains, the vaginal smear permits one, as proved by numerous publications, to detect with high accuracy the cases associated with hormonal disorders. In a series of 397 cases with suspicious clinical signs of threatening abortion the vaginal smear was normal in 107 patients. A careful clinical examination showed that the bleeding was derived from the vagina or the cervix or that the pelvic pains were due to retroflexion of the pregnant uterus or were due to other non-pregnant causes, such as bladder infection or intestinal disorders. In only nine out of these 107 cases did the bleeding have an intra-uterine origin, and the course of the pregnancy revealed the reason: in two cases a hydatiform mole and in seven cases placenta praevia. In another 14 cases, not registered in this series, uterine bleeding was evident, but was due to criminal induction. In some of these patients the vaginal smear contributed to suspecting the real origin of the bleeding, because the smear was normal and no other possible spontaneous cause could be detected by careful clinical examination.

Hormonal bioassays in addition to vaginal smears have been studied by several authors in patients with threatened abortions. From these reports it can be concluded that there generally exists a complete agreement between the hormonal findings and the vaginal smear. This permits the conclusion that if in a case of threatened abortion the vaginal smear is normal, a hormonal cause for the bleeding is unlikely and that an abnormal vaginal smear reflects with a high degree of accuracy hormonal disorders as revealed by bioassays. In some cases with abnormal vaginal smears the hormonal bioassays may be normal. In many of these cases as the pregnancy progresses, the vaginal smear gives more precise evaluation of the hormonal function than the hormonal bioassays. Thus, these cases should be considered as hormonal disorders in spite of apparently normal hormonal findings. This discrepancy can be explained by the fact that the vaginal epithelium is a very sensitive hormone receptor which can indicate hormonal disorders at a level at which they can not yet be detected by the usual hormonal bioassays.

The vaginal smear permits not only the detection of a hormonal cause in threatened abortions, but also permits prognostical deductions. The higher the Eosinophilic Index (together with the corresponding Karyopyknotic Index) the poorer is the prognosis, even under hormonal treatment. If the Eosinophilic Index is higher than 50 in several smears taken at intervals of a few days, the possibility of saving the pregnancy is very small, especially if the pregnancy is older than three months. In the first two months of pregnancy, a particularly high Eosinophilic Index does not necessarily mean a poor prognosis, as shown by Pundel and Van Meensel in 1951. The vaginal smear in entirely normal pregnancies may show at this time rather marked variations of the Eosinophilic Index. But there can be another particular smear type present resembling the postpartum or postabortum type, and composed only of large round or oval parabasal and intermediate cells, some of which show an eosinophilic cytoplasm. If this persists longer than four days, it indicates, in practically all cases, the death of the fetus. This persistent smear type permits avoiding ineffective hormonal treatment which could only unnecessarily delay the expulsion.

D. THE PROGNOSTIC SIGNIFICANCE OF THE VAGINAL SMEAR DURING HORMONAL TREATMENTS FOR THREATENED ABORTIONS

The vaginal smears permit not only selection of the cases in which the possible abortion is related to hormonal disorders, but also permits useful deductions concerning the possible effect of hormonal treatment. As shown by many papers, hormonal treatment can reduce the number of spontaneous abortions, but as experience has proven, there remains a relatively large number of threatened abortions where hormonal treatment could not avoid the expulsion. If hormonal treatment is indicated, the vaginal smear can give, in these cases, important guidance before and during the treatment. Prior to the onset of the treatment, the vaginal smear indicates the degree of seriousness of the case by the degree of the cytological modifications. But as the vaginal smears show only the end result of all hormonal factors, and not the

absolute level of each different sex steroid, it permits no conclusion for the choice of the hormone for treatment. The follow-up study in these cases has shown that regardless of the chosen treatment (progesterone alone, estrogens alone or a combination of both), the chances for saving the pregnancy are good only if the treatment induces a recurrence of the normal vaginal cytology. If the vaginal smear during hormonal treatment shows persistence or even an increase of the abnormal cell pattern, abortion will occur in nearly all cases despite the treatment. Lack of this improvement of the vaginal smear or even increase of the abnormal pattern, such as response to administered estrogens with "estrogenic proliferation" (such as in the non-pregnant woman) can be considered practically definite evidence of death of the fetus (4, 14, 17, 21).

Studies of the vaginal smear prove that the prognosis for the success of a hormonal treatment depends upon the degree of the cytological abnormalities. The best results can be obtained only if the hormonal treatment is started as early as possible when the vaginal smear first becomes abnormal, even in the absence of suspicious clinical signs. Degenerative alterations and even death of the fetus can take place several weeks prior to the appearance of uterine bleeding or uterine contractions. If bleeding has started, the abortion can be avoided only if the placental lesions are not marked and irreversible. Unfortunately, this happens only in some of the cases, while in the remaining cases the fetus is always dead or the ovum has undergone irreversible changes, so that treatment is hopeless. Whatever the treatment, the higher the Eosinophilic and Karyopyknotic Indices are at the onset of therapy, the poorer are the chances to save the pregnancy.

In my material, hormonal treatment was administered in 379 cases. In the first group of 89 patients treatment was started because of abnormal vaginal smears in the absence of actual clinical signs. Only 60 patients (or 67 percent) had a successful pregnancy (49 deliveries at term and 11 premature deliveries), while intra-uterine death of the fetus occurred in eight patients and spontaneous abortion in 21 or 22 percent. Compared with the results of others, this is a very low success rate, but it is not certain that the prescribed treatment was followed correctly by every patient.

Two hundred and ninety patients began treatment while still bleeding or having typical uterine contractions. Treatment (generally with estrogens) was started immediately, but the abortion occurred despite the treatment in 187 patients (or 63 percent). Only 89 patients had a successful pregnancy (32 premature and 57 deliveries at term). In 14 patients, death of the fetus occurred after the sixth month. Success in only 30 per cent is very low compared to the results of others, but this series is not selected. Most of the cases had bleeding for many days and had been sent to the hospital, because the attending doctor considered the abortion as unavoidable. In many of the cases abortion occurred in the first 48 hours after the start of treatment. In all of these cases the vaginal smear indicated poor prognosis before the onset of treatment. Histological examination of the fetus and placenta showed in nearly all cases that the alterations were far too advanced, so that the prognosis was poor from the start. A blighted ovum was found in nearly one-third of these cases. Furthermore, I think that the cytological abnormalities seen in many of the patients of this series were secondary to an organic cause of the abortion, such as uterine fibromas, decidual infections, attempted criminal abortions and uterine malformations. It is evident that such organic causes can finally produce alterations of the placenta with resulting hormonal dysfunction and that hormonal treatment in such cases will be only of secondary importance, because it cannot change the organic cause.

PRACTICAL CONCLUSIONS FOR THE OBSTETRICIAN

Hormonal bioassays are a useful method for the study and diagnosis of hormonal disorders of pregnancy and the control of their treatments. Unfortunately, as shown by large statistical reports, the biochemical assays have a real significance only if they are markedly abnormal and if they can be repeated several times. Furthermore, they are limited to well-equipped laboratories, and the reports can be obtained only after an interval of several days, which may mean loss of precious time. For these reasons they remain the privilege of large obstetrical centers.

Papers published to date clearly show that in a very short time the vaginal smear can give the obstetrician the same information as the bioassay, with the same, if not higher, diagnostic accuracy.

(1) If the vaginal smear is normal in a pregnant patient, she has over 99 percent chance of having a living infant at term.

(2) If the vaginal smear is abnormal in a clinically normal pregnant patient, hormonal treatment should be started immediately, because abortion will occur later without treatment in over 30 per cent of the cases.

(3) Hormonal treatment for threatened abortion is not necessary if the vaginal smear is normal. In these cases a careful clinical examination has to be done in order to discover the possible organic reason for any bleeding or pains.

(4) Hormonal treatment should be administered only in such pregnant patients in whom the vaginal cytology is abnormal. The chosen hormone treatment only has chances for success if the vaginal smear returns to normal. This chance is better the less marked the cytological abnormalities are and the earlier the treatment is started.

(5) Absence of a good cytological response, especially the occurrence of a typical estrogenic epithelial reaction under estrogen treatment, is a very valuable sign for the death of the fetus. The prognostic accuracy of the vaginal smear is higher than that of biological or chemical hormonal assays, since

substitutive hormone treatment can produce pseudonormal hormonal excretion in spite of the death of the fetus, or the biological pregnancy tests can remain positive for a while. It can be concluded that vaginal cytology is actually the best control test, not only for the course of pregnancy, but also for the effect of hormonal therapy of threatened abortions.

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DISCUSSION

JULES-ANDRÉ BRET and FERNAND J. COUPEZ, Paris, France:

We do not have extensive experience with cytology of threatened abortion at this time.

For routine diagnosis of threatened abortion prior to its clinical manifestations our opinion is as follows:

(1) Cytology exhibits pregnancy disorders too late and is not diagnostically specific.

(2) Cytology does not give safe information as to the underlying cause, and cannot replace the classical examinations; on the contrary, it sometimes delays them.

JACQUES FERIN, Louvain, Belgium:

Concerning the prognostic significance of the vaginal smear during hormonal treatment for threatened abortion, it is interesting to point out the following fact: If one orally administers a progestational 19-nor derivative, 17-alpha-methyl-19-nortestosterone (25 mg. daily), for several days to patients exhibiting symptoms of threatened abortion and abnormal estrogenic smears, two changes of the smear may occur:

1) A change to a typical pregnancy smear. The prognosis is then good (43 cases).

2) A change to a smear of postpartum type, with many parabasal cells. The ovum is then dead (7 cases).

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MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil:

It is commonly accepted that high Karyopyknotic Indices in vaginal smears during pregnancy must be regarded as an early, pre-clinical sign of threatened abortion or premature labor. Rogers and co-workers (1) call it a "cytologically threatened abortion."

Furthermore, similar patterns have been observed in early and late toxemia of pregnancy (2), in premature separation of the placenta (3) and in cases of maternal Rh iso-immunization (4). This abnormal picture was also observed by us in three cases of diabetes during pregnancy and in two cases of invasive cervical carcinoma diagnosed close to term.

In principle and for practical purposes we accept the criteria proposed by Pundel and co-workers (5). However, we do not believe that the quantitative estimation of eosinophilic, karyopyknotic superficial cells can give a definite clue for the prognosis in cases of so-called "cytologically threatened abortion," or even in cases with clinical symptoms of abortion. We have seen many cases arriving at a normal term in spite of exhibiting high Eosinophilic and Karyopyknotic Indices, whereas in other instances, patients with lower indices developed clinically threatened abortion or actually aborted.

In our opinion the estimation of the desquamation rate (tendency of cells to crowd together in clusters known as agglutination phenomenon) is at least as useful as the estimation of karyopyknosis and eosinophilia. The decrease or the disappearance of this "agglutination phenomenon," which is accompanied by a decrease of the Döderlein bacilli, regularly precedes abortion and premature labor, as well as normal labor at term.

Relatively abnormally high numbers of eosinophilic, karyopyknotic superficial cells in pregnancy smears must be regarded as a non-specific sign of a disturbance of pregnancy.

They may acquire, however, some prognostic specificity, as Pundel and co-workers first pointed out (5), when they fail to disappear after three or four days of administration of sufficient amounts of ovarian hormones.

Low dosages are useless. We give three to five mg of ethinyl-estradiol (orally) and/or 100 to 200 mg of progesterone intramuscularly.

In the follow-up of patients treated with estrogens for "cytologically threatened abortion," there may be found four main patterns in our daily or weekly smear studies:

A. Persistence of the abnormal smear pattern three or four days after treatment.

B. Partial or total disappearance of the eosinophilic, karyopyknotic cells from the smears and a return to a "normal pregnancy pattern."

C. Appearance of a regressive colpocytological pattern resembling the cytological aspects of the genital crisis of the postpartum period.

D. Appearance of a marked, so called "estrogenic" colpocytological pattern exhibiting up to 90% eosinophilic, karyopyknotic superficial cells.

We have studied 42 patients under hormonal treatment with persistent abnormally high Karyopyknotic Indices between the second and seventh month of pregnancy, with the following results:

1. Thirty women aborted or had premature labor.

2. Ten cases developed clinical symptoms of abortion but carried their pregnancy to or close to term, after several episodes of bleeding and abnormal uterine contractions. Among these ten cases, three cases of placenta previa and one case of fetal malformation were found.

3. The two remaining cases arrived normally at term with persisting abnormally high Karyopyknotic Indices.

An important feature in this group was that the majority of the aborted fetuses were well-preserved and that the immature or premature babies were in most cases born alive or at least with signs of recent death. We believe, therefore, that persistent abnormally high Karyopyknotic Indices are usually associated with abnormal uterine contractibility and an otherwise viable ovum.

In 65 cases with the second type of reaction (disappearance of the abnormal smear pattern) 51 cases arrived normally at term; ten cases developed symptoms coincident with new increases of the Eosinophilic and Karyopyknotic Indices in the first half of pregnancy, but carried their pregnancy to term.

In one case the abnormal colpocytologic pattern, together with vaginal bleeding and cramping, always reappeared with striking regularity when the hormonal treatment was discontinued. This patient had a premature labor. Three patients, however, aborted quite suddenly without noticeable warning symptoms. Vaginal smears were taken 24 to 48 hours before abortion occurred and exhibited the normal pregnancy pattern. It is worthwhile to mention that in two instances the fetuses were reported to have shown signs of life. Another fetus (a five-month fetus) was well preserved, but pathological examination revealed internal hydrocephalus.

The third type of reaction (appearance of a regressive colpocytologic pattern resembling the postpartum pattern) reflects the decrease or the cessation of chorionic activity, and it has been regarded as a sign of fetal death. It is more pronounced in the second half of pregnancy and is not necessarily preceded by abnormally high vaginal cell cornification. We have observed four cases of fetal death where the normal pregnancy pattern of the vaginal smears changed suddenly to a cytolytic pattern, coincident with the cessation of the fetal heart tones; only a short period of time afterwards the smear pattern changed to a postpartum type.

Regressive colpocytologic changes in pregnant patients under estrogen treatment represent the real colpocytologic unresponsiveness to estrogens which is found in the genital crisis of puerperium when the vaginal epithelium suffers marked destructive processes and fails to proliferate (for some time) under hormonal stimulation.

The prenatal death of the ovum determines the onset of the genital crisis, irrespective of the probable retention of the fetus, hence the regressive colpocytologic pattern. The babies, in such cases, are usually stillborn and often macerated.

The regressive colpocytologic changes differ widely. The moment in which they appear in the vaginal smear depends upon the duration of the pregnancy (expressed by the proportion of basal, parabasal and peculiarly rounded, nearly chromophobic and occasionally eosinophilic intermediate cells in the smears). The period of time in which the atrophic picture prevails is directly proportional to the duration of pregnancy. The same rule applies to the time consumed for the regeneration of the epithelium (the genital recovery).

In the first trimester of pregnancy the regressive colpocytologic changes of the "post abortum" or prenatal death of the ovum are very slight compared to the genuine atrophic postpartum changes at term or in the prenatal death of the ovum near term. The genital recovery in the first trimester of pregnancy, following abortion or intra-uterine death of the ovum, is often so rapid that if the patient is under estrogen treatment, one finds suddenly a marked "estrogenic" smear pattern in cases of missed abortion. This represents the meaning of the type D reaction: appearance of a marked "estrogenic" smear pattern exhibiting up to 90% eosinophilic, karyopyknotic superficial cells.

We have studied the colpocytologic aspects in 120 cases of clinical suspicion of fetal death. In 37 cases the cytological examination was handicapped by infection and only in 30 cases, with marked regressive smears, was a diagnosis of fetal death made at the first examination. In this group one immature baby was, nevertheless, born alive. In 18 cases, ranging from the fifth to the eighth month of pregnancy, where at the time of the first colpocytologic examination the fetuses were most probably already dead, we did not find clear regressive cytologic changes. The expected changes appeared, however, sometime later in 11 patients, when we were able to follow-up with daily smears. In this group five patients were treated with estrogens.

Fetal death, therefore, is not necessarily coincident with the marked decline or cessation of chorionic activity, which is responsible for the so-called normal cytologic pregnancy pattern. This is what happens also in cases of hydatiform mole where the vaginal smear may show an absolutely normal pattern.

The appearance of a regressive colpocytologic pattern in patients treated with estrogens for "cytologically threatened abortion," type C reaction, is uncommon in cases which did not develop clinical symptoms during the follow-up.

It is our belief that one can only make safe diagnoses of fetal death with this cytologic pattern if it fails to change to a normal pattern during three or four days of continued estrogen administration. We have seen cases which were clinically diagnosed as threatened abortion, with regressive smears of the postpartum type, which later developed to term.

The fourth type of reaction (appearance of a marked, so-called "estrogenic pattern") indicates complete loss of the pregnancy pattern of the smear. We have seen this reaction only in the first trimester of pregnancy. In 53 cases all aborted or had missed abortions.

We agree with the main speakers that vaginal cytology is a good prognostic method in several pregnancy disorders which can hardly be surpassed by hormonal assay. We agree, furthermore, that vaginal cytology yields a high prognostic accuracy when used to test the responsiveness of the vaginal epithelium to estrogens.

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JOSÉ MARIA MEZZADRA and GUILLERMO TERZANO, Buenos Aires, Argentina:

We can state that the cytological pattern of vaginal smears permits the assessment of normal pregnancy in a large percentage of cases. Apart from sporadic increase of the number of eosinophilic cells (about 20 to 25%), when there is no infection, changes in the cytological picture of the smears usually indicate abnormality.

During the first trimester, a high Eosinophilic Index together with uterine cramps and the presence of erythrocytes in smears, allows one to predict an abortion. In these cases the presence or absence of navicular cells is an indication of hormonal deficiency.

An Eosinophilic Index above 25 with a Karyopyknotic Index above 50 should be considered a danger signal and an indication that the patient must be given hormonal treatment.

If after estrogen therapy the amount of eosinophilic cells decreases, the prognosis should be good, if not, progesterone should be administered. The persistence of high Eosinophilic and Karyopyknotic Indices after estrogen and progesterone therapy is a good reason for a poor prognosis, and abortion is to be expected. The patient must be carefully watched until the Eosinophilic Index returns to normal.

In those cases of missed abortion high Eosinophilic and high Karyopyknotic Indices, absence of navicular cells and the presence of postpartum type cells and erythrocytes are reliable signs.

HERBERT E. NIEBURGS, New York, New York, U. S. A.:

The role of cytology in the prognosis of disorders during pregnancy is excellently outlined by the contributions of Pundel, Gaudefroy and Montalvo-Ruiz. The statistical evaluation by all three authors indicates the higher rate of pregnancy salvage when threatened abortion was diagnosed by routine vaginal smears with immediate institution of therapy. The greater sensitivity of the vaginal epithelium to hormonal changes than that of hormone bioassay's is clearly evident from the papers presented. I agree with Pundel that the evaluation by general morphology of the cells is less satisfactory than considering the Karyopyknotic Index. As stated in other discussions, the evaluation of the Eosinophilic Index may be misleading since this depends on a number of variable factors such as preparation and variation of stain, pH, infections, etc. A precise description of the morphology of the cells, which, in addition to the karyopyknotic cells, is of importance in the diagnosis of hormonal disorders during pregnancy, should present more objective means of diagnosis. The recommendation not to administer hormonal therapy, in the presence of threatened abortion when a normal vaginal smear is found, presupposes a degree of accuracy for this method far beyond that reported by most authors.

ERICA WACHTEL, London, England, U. K.:

While I fully agree with what has been said so admirably by the speakers on this subject, I would like to ask them a question about what they have not said. There is hardly any doubt any more that vaginal smear patterns during pregnancy express existing hormonal disorders in a high proportion of cases and that these disorders are often correctly interpreted. What is being diagnosed, however, is a disturbed balance between estrogen and progesterone.

Smears obtained during hormonally normal pregnancies show less estrogenic effects than smears collected in the proliferative phase (low Karyopyknotic and Eosinophilic Indices) despite higher estrogen production, because the effects of estrogen are mitigated by high progesterone effects. Where, however, there is a progesterone deficiency, the unopposed action of estrogen will become manifest by a rise in the Karyopyknotic and Eosinophilic Indices, in addition to the direct evidence of diminished progesterone activity, such as decrease in clumping and decrease in navicular cells. This mechanism is logical and clearly understood. I would, however, like to ask the authors of the above papers by what cytological criteria they diagnose estrogen deficiency while the ovum is still alive, that is to say, in what way do smears in pregnancy, with deficient estrogen but adequate progesterone production, differ from normal pregnancy patterns?

CLOSING REMARKS

MARCEL GAUDEFROY:

I thank the discussants very much for their remarks. I would like to answer Erica Wachtel's question: "In what way do smears in pregnancy, with deficient estrogen but adequate progesterone production, differ from normal pregnancy patterns?" In my opinion, the diagnosis of estrogen deficiency with normal progesterone level is very difficult and demands a great deal of experience. Many times, in smears with a normal progesterone pattern in some places, and outer intermediate cells without curled borders and without clumping in other places, and also containing inner intermediate cells which are non-navicular and not of the oyster type, I suspected the above condition, but affirmed it only after hormonal

administration. For this delicate diagnosis, perfect technique is necessary, and also one must know if the smear is from a pregnant woman, because in that case the smear pattern is very close to that of hypohormonal amenorrhea.

LUIS MONTALVO-RUIZ:

To Erica Wachtel: In a pregnancy with a living ovum there cannot be an estrogenic deficiency; therefore, the smears must have the normal cytological pattern.

To Ferin: I agree that smears indicate whether or not the prognosis is good, no matter what the therapy may be.

To Mezzadra and Terzano: Of course there can be cases in which the Eosinophilic Index rises without existing infection. It can be caused by drying the smear before fixation, changing the pH of the fixative, or it can be due to prolonged differentiation. The above can change the Eosinophilic Index but not the Karyopyknotic Index. It is more valuable when both indices are elevated.

J. PAUL PUNDEL:

I thank the discussants for their interesting contributions to this important cytological and obstetrical problem, and I must say that I agree completely with Erica Wachtel, Nieburgs, Ferin, Mezzadra and Terzano, and on most points with Kamnitzer.

The only disagreement that I found was the statement of Bret and Coupez, so I would like to begin my closing remarks with some objections to their remarks. I think that today it is well-known that all treatment for threatening abortion has the maximum chance of success only if it is started as early as possible and that in many cases where bleeding and uterine contractions have started, treatment arrives too late. It is the practical advantage of vaginal smears to permit a systematic control of every pregnant patient, with the same ease as the control of urine and blood pressure, in order to find out at the earliest possible moment the possible aborters and to permit, therefore, treatment as early as possible. Therefore, I cannot follow the discussants in their first statement, and I would like to ask them by what other systematic test do they hope to determine the hormonal disorders before the appearance of clinical signs. By systematic hormonal assays? Unfortunately, I do not have sufficient experience with hormonal assays during pregnancy, because in my country most patients and social insurances do not have enough money to permit systematic hormonal assays. Indeed, if hormonal assays (the research interest of which is beyond question) are to be of practical value, they need to be repeated and to be as complete as possible. How many laboratories would be able to assume systematic hormonal assays, and how much would the cost be of such systematic controls? To the second objection of the discussants, I would reply that they can find sufficient explanations and details in the main papers. I hope, however, that if Bret and Coupez in the future have the same experiences as the main speakers, they will change their statements.

Erica Wachtel asks how it would be possible to "diagnose estrogen deficiency while the ovum is still alive, that is to say, in what way do smears in pregnancy, with deficient estrogen but adequate progesterone production, differ from normal pregnancy patterns?" As correctly stated by Erica Wachtel, the vaginal smear gives only the end effect of the combined vaginal action of estrogens and progesterone, so that it is not always possible to obtain quantitative indications concerning the separate action of these hormones. Therefore, minor estrogen deficiencies with normal progesterone production will not produce evident changes of the normal vaginal smear. Marked estrogen deficiency with normal progesterone production is rare, and I think that up to a certain degree, which I cannot express in biological units, the vaginal smear will remain normal. But in some cases which were correlated with hormonal assays, I have seen a real regressive type of vaginal smear, with the disappearance of the navicular cell clusters and the appearance of some parabasal cells, similar to the smears observed after isolated administration of progesterone to castrated women.

Estrogen treatment in all of these cases, observed between the third and the fourth month of pregnancy, produced a rapid return to a normal pregnancy smear. As patients with biochemically-proven estrogen deficiency and fairly normal progesterone production, but with normal pregnancy smears, as well as other patients who returned to normal under estrogen treatment after previous regressive smears (all had successful pregnancies or presented no serious clinical symptoms) I think that the prognosis for these patients, in general, can be considered as good, and better than those patients with progesterone deficiencies. It is interesting to note (what Ferin has pointed out and what I could myself observe) that progesterone or synthetic progesterone-like substances do not produce evident changes in the normal vaginal cytology, even if they were administered in massive dosage, so that they should have produced an important predominance of progesterone in the estrogen-progesterone balance. Only if the ovum is dead or has undergone irreversible regressive changes, will progesterone produce vaginal changes similar to postpartum cytology. Therefore, I continue to believe that as long as the vaginal smear is normal, a dangerous disorder in the balance between estrogens and progesterone can be eliminated without serious risks for the patients and that hormonal treatment is not absolutely necessary. That is my experience, gained from many thousands of pregnancies controlled by vaginal smears. But as in mankind and in medicine, most extraordinary things are possible; so please do not consider my statement as dogma.

To Kamnitzer: I am rather surprised at so many cases of symptomless pregnancies with persistently high Eosinophilic Indices. In my own material such cases occurred only twice in 3,000 pregnancies. Both patients had previous abortions. Hormonal treatment was refused in both cases for fear that in case of success the baby might present malformations, but as the patients observed bed rest and

had some sedative treatment, I would not make any conclusions in these cases. I would like to ask Kamnitzer if in all his own cases the smears were taken only from the lateral vault of the vagina, after insertion of a speculum and before any gynecological examination, and if vaginal douches could be excluded. In cases where these precautions had not been observed, I also found many cases with abnormally high Eosinophilic Indices, but correct control smears always showed normal smears with the two referred exceptions.

I agree that there exist pregnancies with rather low, but abnormal Eosinophilic and Karyopyknotic Indices which can present clinical symptoms or even can have abortions. But abortions with entirely normal vaginal smears also exist as I stated in my paper, and even if they are rare, they should remind us that not all abortions have a hormonal cause.

Kamnitzer gives reference to abnormal cytological findings in toxemias, diabetes, abruptio placentae and even in cases of Rh-iso-immunization. A priori, as long as such troubles are not accompanied by troubles of the estrogen-progesterone balance, and this is unlikely, at least for the Rh-immunization, we should not expect to find abnormal vaginal smears just because the patient presented these complications. In over 200 of these cases I have found nearly all of them to have entirely normal vaginal smears, with only 10 or 15 exceptions, resulting from a secondary alteration of the placenta accompanied by fetal distress or death. If the vaginal smear presents changes, they are related only to regressive changes of the placenta, and they can not be used for the diagnosis of preeclampsia, diabetes or impending abruptio placentae, or even for the evaluation of the seriousness of these complications or for Rh-iso-immunization. Let us not ask too much from the vaginal smear and let us apply it only where it has a serious biological basis.

The regressive smear pattern, especially under estrogen treatment as pointed out by Kamnitzer, is a valuable test for the diagnosis of fetal death or serious fetal distress, as this negative estrogen effect is directly proportional to the duration of pregnancy. Here I agree completely with Kamnitzer, but I would add that as shown by myself and others, during the first three or four months of pregnancy a positive or normal estrogen response, as in the nonpregnant woman, also is a very sensitive test for the death of the ovum. In these cases the post partum (or better the post abortum), with its particular hormonal reactions, is too short to produce a vaginal inertia against estrogens which, therefore, rapidly produce the same vaginal reactions as in the nonpregnant woman.

For general conclusions and for the cytological patterns observed in cases of intra-uterine death of the fetus, I completely agree with Kamnitzer.

The vaginal smear, as shown by all speakers, has now proved its practical value for the control of the hormonal balance during pregnancy and for the early detection of these troubles long before the appearance of the clinical symptoms, thus permitting treatment at the earliest possible moment and with a maximum chance of success. This is an important conclusion which permits us to accept some minor differences between several authors, disagreements which are only of theoretical interest and do not influence the practical results of the vaginal smear during pregnancy.

EFFECT OF ADMINISTERED ESTROGENS ON THE VAGINAL EPITHELIUM DURING PREGNANCY AND THE POSTPARTUM PERIOD

MARIO de BENNING KAMNITZER
Rio de Janeiro, Brazil

In pregnancy, estrogens fail to induce the appearance of eosinophilic, karyopyknotic vaginal cells. This seems to happen with endogenous estrogens and most certainly with administered estrogenic hormones (1, 2, 3, 4, 5, 6, 7, 8, 9, 10). A similar change of the usual response to estrogens was shown by Zondek and co-workers (11), concerning the crystallization phenomenon of the cervical mucus in pregnancy (12).

In a group of 20 patients in the second or third trimester of pregnancy, all of them exhibiting normal vaginal smears, we administered the following estrogenic compounds:

Ethinyl-estradiol - 3 mg daily per os.

Estradiol dipropionate - 5 mg as weekly intramuscular injections.

Estradiol monobenzoate - 10 mg as monthly intramuscular injections.

In all cases treated with estrogens the vaginal smears did not show any significant increase of the Karyopyknotic Index (it must be borne in mind that in the first trimester there are usually oscillations of the Karyopyknotic Index) but some developed marked cytolysis. In other cases which exhibited cytolysis in previous smears, there were sometimes no changes at all or sometimes gradual disappearance of cellular lysis. We are so far unable to understand the meaning of these changes. Nieburgs and Greenblatt (13) seem to believe that cytolysis in pregnancy smears reflects an increase of estrogenic activity; meanwhile Koller and Artner (14) adopt an opposing point of view. On the other hand, we have observed that administered estrogens tend to inhibit the previously existing presence of eosinophilic, karyopyknotic cells (2, 5, 6, 7).

One might ask, if in pregnancy the Karyopyknotic Index does not reflect a decrease of estrogenic influence.

Administered estrogens, particularly ethinyl-estradiol, are able to prevent the appearance of the slight, regressive changes of the last weeks of pregnancy, but they do not seem to have any influence at term on the peculiar cytologic changes coincident to the so-called ripening of the cervix (7, 15).

In the postpartum period one must distinguish two phases: (a) the genital crisis, (b) the genital recovery (15). During the genital crisis the vaginal smears are non-responsive to estrogens after delivery and failed to induce a colpocytologic response. The same hormone, when given during the last month of pregnancy, seemed to show a remarkable influence on the genital crisis, which was definitely less severe than normal (15).

The usual (non gravid) response of the vaginal epithelium returns only after completion of the regressive processes of the genital crisis (2, 15), which lasts from 2 to 3 weeks after full term delivery.

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J. PAUL PUNDEL

Luxembourg, Luxembourg

The vaginal effect of estrogens administered during pregnancy was first studied in 1949 by Van Meensel in the laboratory of Bourg in Brussels, and nearly at the same time by Gaudefroy in Lille. These first findings have been completed in a monograph by Pundel and Van Meensel in 1951 and later confirmed by others (1, 2, 4, 9).

A. THE VAGINAL EFFECTS OF ADMINISTERED ESTROGENS DURING THE NORMAL PREGNANCY

In general, during normal pregnancy estrogens produce no typical estrogenic reactions in the vaginal epithelium and cytology. The qualitative reactions are nearly the same during the entire course of pregnancy, but there exist some quantitative variations in the vaginal response to estrogens when they are administered in the first trimester, the last two trimesters or in the two weeks preceding the delivery at term.

1. Effect during the last two trimesters or the placentar phase of the pregnancy

The administration of estrogens during this phase of a normal pregnancy has no specific estrogenic effect upon the vagina, if the treatment is given per os or by parenteral injections. The estrogens do not influence the Eosinophilic and Pyknotic Indices, which remain at the same normal level during the administration of estrogens, even when they are administered in massive dosage. With Van Meensel, I have studied over 40 cases of normal pregnancies receiving estrogens, and the dosage administered varied from 10 to 300 mg of dienestrol, diethylstilbesterol or 50 to 2000 gamma of ethinyl-estradiol per os daily over several weeks. The only modification which could be seen in the vaginal cytology during this treatment was a disappearance of the typical thick clusters, so that the vaginal navicular cells appeared more often as individual cells. The Pyknotic Index also showed an occasional rise but without any rise of the Eosinophilic Index. The variations of the Pyknotic Index appeared only if the dosage was very high and the pregnancy was at the beginning of the fourth month or near term. In patients with a previous cytolytic smear the administration of estrogens was followed in some cases by a regression of the cytolysis. This experimental administration of estrogens during normal pregnancies had no harmful effect upon the progress of the pregnancy or in the development of the baby.

2. Effect of estrogens during the first trimester of pregnancy

The vaginal reactions during estrogen administration in the first trimester are, in general, the same as in the latter part of pregnancy, but only if the administered dosage is not too high (less than 100 mg of dienestrol of diethylstilbestrol per day). However, occasionally the vaginal cytology responds with a rise of the Eosinophilic and Pyknotic Indices, if the estrogens are given in very high dosage. This rise of the E.I. and P.I. is never as marked as after the administration of the same dosage of estrogens to a non-pregnant woman, and if the administration is continued over the third month, the Eosinophilic and Pyknotic Indices return to normal; the estrogens no longer give any estrogenic effect on the vaginal cytology. The luteal reactions (such as cell folding, curling, shedding in clusters), in general, are not influenced by estrogens, even if they induce a rise of the E.I. and P.I.

3. Effect of estrogens in the last two weeks before delivery at term

Shortly before delivery, the administration of estrogens again induces some estrogenic reactions, shown as a rise of the Eosinophilic and Pyknotic Indices, but this partial return to a normal estrogenic reaction of the vagina appears only if the pregancy is at term. In questionable cases the administration of

estrogens can, therefore, be used as a valuable test in order to find out if the pregnancy is really at term (6, 7).

Conclusions

There exists a complete agreement among all authors that during pregnancy the vaginal cytology does not respond in a normal manner to administered estrogens, as it does in the non-pregnant woman. The reasons for this absence of specific vaginal reactions and the exceptions that occur at the beginning and the end of pregnancy have been studied in a previous symposium of the Academy and will be discussed shortly in the next part of this paper. There exists no significant differences in the vaginal response, whether the administered estrogens are synthetic or natural, but this negative behavior of the vagina exists only if the estrogens are administered per os or parenterally, while the reactions after local application of estrogens are different (5).

B. EFFECT OF ADMINISTERED ESTROGENS IN PREGNANCIES WITH HORMONAL DISTURBANCES

It is now well-known that in pregnancies with hormonal disturbances predisposing to premature interruption, the vaginal smear shows, in nearly all cases, an abnormal picture. There is a decrease of the navicular cell clusters and an increase of the superficial cells, which appear more as single and flat cells. Simultaneously, the Eosinophilic and Pyknotic Indices rise to abnormally high levels. It appears that these cytologic modifications in an abnormal pregnancy indicate, at first, only a regression of the progesteronic activity of the corpus luteum or placenta, so that the estrogens remain the predominant factor in the determination of the cytologic picture.

If, as is agreed by several authors (1, 2, 3, 4, 5, 8, 9, 10), estrogens are administered to such patients, they induce a particularly interesting vaginal reaction supporting the theoretical basis for the value of estrogens in the treatment of impending abortions. The first response appears in the Eosinophilic Index which returns progressively to a more or less normal level and is followed by a decrease of the Pyknotic Index. The navicular cells reappear and the smear presents, after a while, again a more or less normal picture. Only in the first trimester of pregnancy can estrogens accentuate the abnormally high Eosinophilic and Pyknotic Indices; however, the smear does show a return to a normal luteal picture by the reappearance of the typical thick cell clusters and the folding and curling of the cell cytoplasm, so that the abnormally high Eosinophilic and Pyknotic Indices are the only abnormal elements. But this partial estrogenic reaction appears only if the administered dosage of estrogens is unnecessarily high (over 100 to 200 mg. of dienestrol per day), and the lowering of the dosage will be followed by a return of the smear to within normal limits.

Conclusions

During an abnormal pregnancy, the estrogens produce a paradoxical luteal effect in the vaginal cytology, as their administration is followed by the disappearance of the abnormal estrogenic reactions and a return of the typical luteal modifications, such as cell clusters, folding and curling. But this progesterone-like effect of estrogens exists only if the pregnancy can be saved. From several reports it can be concluded that a normal estrogenic vaginal reaction (rise of the Eosinophilic and Pyknotic Indices with the disappearance of the cell clusters and the cell folding) after the administration of estrogens in abnormal pregnancies, can be used as a valuable sign for the death of the fetus.

C. THE BIOLOGICAL MECHANISM OF THE NEGATIVE AND PARADOXICAL LUTEAL EFFECT OF ESTROGENS DURING PREGNANCY

It has been shown that the negative response of the vagina to estrogens during pregnancy cannot be considered a true physiological state (where the vaginal epithelium does not respond in a normal manner to estrogens), since the local administration of estrogens produces the typical estrogenic reactions in the vaginal smear, as in the case of the normal non-pregnant woman (5). From these findings and from the paradoxical progesterone-like effect of estrogens in abnormal pregnancies, it can be concluded that during pregnancy, estrogens, after either oral or parenteral administration, act upon the vaginal epithelium not as estrogens, but as progesterone or progesterone-like substances. This theory could explain the negative effect of estrogens in normal pregnancies, since here the vaginal smear shows a maximal progesterone effect which cannot be accentuated either by estrogens or by progesterone.

It appears that the estrogens administered are converted in the organism into progesterone-like substances, but up to today we have no exact scientific arguments concerning the chemical process by which this transformation occurs. Nevertheless, from the cytological findings we can suggest some arguments that the organ responsible for this conversion of estrogens to progesterone could be the placenta:

1. The most evident negative effect is observed during the last two trimesters of the pregnancy with the exception of the last two weeks. This is the phase where the placenta has reached its maximal development.

2. The most evident paradoxical luteal effect of estrogens is observed only in pregnancies which can be saved, where the placenta has not yet undergone irreversible regressive changes.

3. If the placenta has lost its vitality in an irreversible manner, estrogens immediately induce normal estrogenic reactions in the vaginal epithelium.

The fetus seems not to partake in this mechanism, since the same negative or paradoxical effect of estrogens has been observed in pregnancies in which the death of the fetus occurred after the sixth month, followed by spontaneous delivery at normal term, with a rather normal placenta, and in cases of hydatiform moles without any fetus (own observations).

This theory of the placental site of the transformation of the administered estrogens into progesterone-like substances could also explain the partial estrogenic reactions of estrogens shortly before term and when administered in high dosage during the first weeks of pregnancy. It is known that the vitality of the placenta decreases before delivery and that in the first weeks of pregnancy the placenta has not yet obtained its full development. One could suppose, therefore, that in these cases the placenta is no longer or not yet able to assume the complete transformation of all of the administered estrogens into progesterone-like substances, so that at least part of these estrogens can act upon the vagina as estrogens in the same manner as in the non-pregnant woman.

D. VAGINAL EFFECTS OF ESTROGENS ADMINISTERED DURING THE POST PARTUM

The vaginal reactions to estrogens during the postpartum period have been studied by several authors, and particularly in one of the past symposia (Vol. II, No. 2, 1958), so that it seems unnecessary to present the findings in detail in this paper. The problem can be summarized as follows: There exists a complete agreement that the vaginal epithelium does not respond in a normal manner to estrogens during the immediate postpartum period. However, it appears that this vaginal inertia to estrogens exists only for estrogens administered orally or parenterally, while the local application of estrogens produces a rather normal estrogenic reaction. The mechanism of this particular form of hormonal inertia has still to be studied.

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DISCUSSION

JACQUES FERIN, Louvain, Belgium:

The conversion of chemically, very different estrogenic substances, such as dienestrol, diethylstilbestrol, and ethinyl-estradiol, into progesterone seems very unlikely. I think that we must suppose another mechanism for explanation of the paradoxical effects of estrogenic treatment upon vaginal smear and urinary titer of pregnandiol: the estrogenic compounds increase the blood flow to the uterus, and, in the uterus, to the placental area; the secretory activity of the trophoblastic cells of the chorionic villi is therefore stimulated. It should be interesting to study the effect of estrogens upon the gonadotropine titer.

MARCEL GAUDEFROY, Lille Nord, France:

There is almost nothing to add to Pundel's description and on all the points my experience confirms his own.

Nevertheless, I would like to point out one observation on the activity of moderately administered estrogens during the first three months of normal pregnancy. In many cases, 25 or 50 mg of stilbestrol or 500 - 1000 gammas of ethinyl-estradiol have in two weeks modified the smear pattern, which became similar to the one of advanced pregnancy.

I also agree with Kamnitzer, and I have likewise observed that administered estrogens produced sometimes, but not always, gradual disappearance of cytolysis.

FRANTIŠEK HORÁLEK and MOJMÍR SONEK, Brno, Czechoslovakia:

In accordance with Pundel's and Kamnitzer's findings we found a negative estrogenic effect during pregnancy. We make use of this phenomenon, which we consider to be specific, for the diagnosis of early pregnancy.

Similarly, we make use of the fact that positive estrogenic effect occurs in pregnancies at term, as stated by Pundel. In the differential diagnosis of primary, weak, uterine contractions for premenstrual pain, we use simultaneously the therapeutic effect of estrogens (10 - 20 mg) to intensify the contractions. A warning should be noted in the use of the large doses, since the onset of lactogenesis could possibly be delayed.

We have, however, certain doubts concerning Pundel's explanation of the biological mechanism of negative estrogenic effect during pregnancy. Even if the possibility of the conversion of natural estrogens into progesterone-like substances (on the basis of the similarity of their chemical structure) could be admitted, it cannot be accepted in the case of diethylstilbestrol. After its application we also find negative estrogenic reaction; also its chemical structure differs entirely from that of all natural steroids.

Neither can the further theoretical supposition, based on the commonly accepted theory concerning the conversion of estradiol to inactive estriol during pregnancy as an effect of progesterone (1), explain the negative effect of stilbestrol.

We are led to the hypothesis that the characteristic signs of pregnancy in cytology are not only the result of a decreased level of estrogens, but rather a result of the active influence of both estrogens and chorionic gonadotropins. We take a similar view of the cytological pattern of the puerperium, where we consider the cells of lactation to be the result of the joint activity of estrogens and prolactin with which the gonadotropins are closely related. The morphological and cytochemical character of the cells of lactation and the navicular cells distinguish them from the usual parabasal and intermediate cells and leads us to this opinion (2).

We consider negative estrogenic reaction to result from an absolute predominance of chorionic gonadotropins and prolactin. As a result of this predominance, neither natural nor artificial estrogens can manifest themselves in the cytological picture.

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LUIS MONTALVO-RUIZ, Madrid, Spain:

We agree with Pundel's and Kamnitzer's observations concerning estrogen administration during the first trimester of pregnancy.

We did not find any variation in smears after administration of a high dosage of estrogen from the 4th to the 9th month of pregnancy. The same thing has been found by Raucher (6) after injecting 5000 mg of estrogens over a period of 10 days.

We have injected estrogens in 20 women in their late stage of pregnancy. Twelve of them had prolonged pregnancies. The results agree only partially with those of Pundel. In 60% of the cases there was an increase of the Eosinophilic and Karyopyknotic Indices. In the remaining 40% we did not observe any variation even when we injected 300 mg of diethylstilbestrol within 6 days to one part of the group and 500 mg of diethylstilbestrol within 6 days to the other part. Our observations regarding estrogen therapy in pregnant women with hormonal disturbances fully agree with Pundel's findings. We presented this matter at the Annual Congress of Spanish Gynecologists in Malaga in December, 1957.

We agree with Pundel that the placenta is the place where alterations producing paradox responses take place. However, we do not believe that these take place by transformation of estrogens into progesterone or progestational substances. It is difficult to admit that estrogens of the stilbene group, like the diethylstilbestrol, so frequently used, can be transformed into progesterone or progestational substances, the chemical structure of which is very different: The progesterone molecule has the cyclopentanophenanthrene ring from which all sex steroids are derived, while the chemical structure of the synthetic estrogens is entirely different. One could admit the possibility that ethinyl-estradiol, in spite of being a synthetic estrogen, could be transformed into progestational substances since it has the estrane ring. For this same reason the naturally occurring estrogens (estrone, estradiol and estriol) also might be transformed into progestational substances. We know after Smith and Smith (7) that the placenta takes the estradiol and transforms it into estrone and estriol, in which form it is eliminated through the urine (8). We know too that the overload of estradiol in the pregnant woman is followed by a quick disappearance of this substance in the blood (4), because the placenta destroys and inactivates it. Oneson and Cohen have demonstrated that this breakdown of estrogens occurs in the placenta under the influence of progesterone. Following the above statements, we can admit that the placenta destroys the estrogens, but no one has ever demonstrated that estrogens can be transformed into progesterone.

We think cytological changes during hormonal disturbances of pregnancy such as threatened abortion are due to alterations or lesions of the trophoblastic villi, since it has been shown (9, 11) by his-

tochemical studies, that the secretion of the gonadotropins takes place in the Langhans' layer. These workers, as well as Wislocki and Bennett (10), have been able to demonstrate the formation of steroids in the syncytial layer. Ashbel and Seligan (1) also have found steroids with carbonyl groups in the syncytium. Thus, lesions of these trophoblastic villi could cause a deficiency or lack of progesterone which subsequently could not act upon the estrogens. The estrogen would thus remain in the maternal blood in a greater amount and would consequently cause an increase of the Eosinophilic and Karyopyknotic Indices.

If we give estrogen therapy in such conditions, there will be an increase in the progesterone and gonadotropin secretion by the chorion (3), re-establishing the hormonal equilibrium by action of the estrogens upon the chorion which now secretes more progesterone, but not by transforming estrogens into progesterone since we are using the stilbene type of estrogens.

Moreover, it we consider the luteotropic action of the chorionic gonadotropin, which also increases under stilbene administration (2), one can better understand the effect of estrogens on hormonal disturbances and also cytological changes during pregnancy.

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CLOSING REMARKS

MARIO DE BENNING KAMNITZER:

It is commonly supposed that the normal colpocytologic pregnancy picture and the so-called paradoxical response to estrogens are due to an overwhelming progestational influence. This seems plausible, but it has not been proven.

In two castrated women, after a previous priming with estrogens, we administered daily injections of 100 mg. progesterone for 8 days.

From the fifth day on, we obtained a colpocytologic picture which seemed identical with the pattern of advanced normal pregnancy. On the sixth day we administered 0.1 mg ethinyl-estradiol, and two days afterwards a definitely recognizable estrogenic reaction appeared in the smears.

We realize that this experiment does not prove anything except that in the mentioned experimental pseudopregnant vaginal conditions, progesterone was unable to prevent the appearance of an estrogenic reaction.

It really seems curious that the characteristic cytologic picture in pregnancy, and also the paradoxical response to estrogens, must have their cause in the ovum, for in nonpregnant conditions the colpocytology is different.

We think that any hypothesis presented to explain the paradoxical reaction of the vaginal smear to estrogens in pregnancy should also explain the paradoxical reaction of the cervical mucus, the latter reaction is reportedly observed from the very early stage of pregnancy, even before the appearance of amenorrhea, because at that stage even the sensitive Ascheim-Zondek reaction may be negative.

The hypothesis of Horálek and Sonek is interesting, but it seems that the common appearance of a vaginal cornification peak precisely at the time of maximal gonadotropin excretion does not support their theory.

It seems to us that in the present state of knowledge of gestative endocrinology, theoretical reasoning concerning colpocytology stands on a firmer ground when it deals with strictly phenomenologic observations, as Ferin points out in his discussion.

J. PAUL PUNDEL:

It has been a pleasure for me to read the discussions and I thank the authors for their interesting comments.

I am glad to see that the cytological findings of the main speakers and discussants are completely uniform concerning the vaginal effect of estrogens during normal, as well as disturbed pregnancies. I also

agree with Kamnitzer and Gaudefroy that estrogens sometimes, but not always, produce a gradual disappearance of cytolysis. To Montalvo-Ruiz I would like to ask the type of vaginal smears that his 20 patients had before the administration of estrogens. As long as the patients show normal pregnancy smears of the type "before term," a positive estrogen effect is unlikely to occur, and delivery, in general, will not happen within the next five days. The exceptions are not more than 10 per cent and can be explained by the argument that the spontaneous onset of labor does not always have a hormonal cause, since it is known that purely mechanical modifications of the uterus can also start labor.

My hypothesis concerning the possible etiological mechanism of the paradoxical progesterone effect of estrogens is based upon two empirical facts: (1) local administration of estrogens during pregnancy produces a normal estrogenic response in the vaginal epithelium, (2) the progesterone reaction of estrogens is observed only if the estrogens are administered orally or parenterally, and if there exists a placenta which has conserved its hormonal vitality. Therefore, one could suppose that the administered estrogens could be transformed in the placenta into progesterone-like substances or that they could stimulate the secretion of progesterone.

The first hypothesis seems, as pointed out by the discussants, unlikely, because important differences exist in the chemical structures of progesterone and several synthetic estrogens. Therefore, I must confess that after the pertinent comments of the discussants I now prefer the second hypothesis: stimulation of the progesterone production in the placenta by estrogens whose excess could, furthermore, be destroyed or inactivated in the placenta, the latter also explaining the negative estrogen effect of the oral or parenteral administration.

Drs. Horalek and Sonek consider the negative estrogen effect to result from an absolute predominance of chorionic gonadotropins and prolactin. Indeed, it appears that the estrogens stimulate not only the placental secretion of progesterone but also that of chorionic gonadotropins, as pointed out by Ferin and Montalvo-Ruiz. But this theory seems, a priori, unlikely, at least for the negative estrogen effect during pregnancy, because it has been proved that the administration of 4,500 I. V. of chorionic gonadotropins produces no evident changes in the vaginal cytology of castrated women (1) and that larger dosages (5,000 - 20,000 I. V. daily) can produce estrogen-like reactions (2, 3). However, these could be the result of stimulation of the adrenal cortex and not a direct effect of the chorionic gonadotropins upon the vagina. For these reasons I do not think that a high level of chorionic gonadotropins could be the cause of the negative estrogen effect during pregnancy. The two following biological facts are in contradiction with this theory: (1) The complete negative estrogen effect is obtained only after the third month of pregnancy, when the level in the blood is rather low; also during the first two months, when chorionic gonadotropin estrogens sometimes produce a rather normal estrogen effect, the chorionic gonadotropin level is the highest. (2) A positive estrogen effect occurs after local administration of the estrogens.

There remains the possible interference of prolactin. We know that prolactin secretion is very low during pregnancy and that estrogens can block lactogenesis. But, as the endocrinologists continue to discuss whether or not the estrogens have a stimulating or a blocking effect upon prolactin secretion in the human female, we remain in a terrain where our exact biological knowledge concerning the estrogen-prolactin relationship in the human female is rather small. I think, therefore, that the hypothesis of a blocking effect by chorionic gonadotropins or prolactin as the explanation for the negative estrogen effect during pregnancy is not yet supported by any positive argument, while the other theory, of the stimulation of progesterone secretion in the placenta and inactivation of excess estrogens by the same placenta, seems the most likely. Nevertheless, we can accept this theory only for the negative estrogen effect during pregnancy, and there remains an explanation for the negative estrogen effect during the post partum, where the placenta has to be excluded as a possible factor. But a possible interference by prolactin, as supposed by Horalek and Sonek, merits interest for the post partum, and since we still know nothing about the possible vaginal effect of prolactin on the human female, this would be a very interesting subject for further research.

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VAGINAL CYTOLOGY AT THE END OF PREGNANCY

CAMILLE J. P. LICHTFUS
Athus, Belgium

Recent publications have shown that the vaginal smear is a very helpful clinical test at the end of the pregnancy (7 - 30). These conclusions are based upon the observation of some particular modifications of the vaginal smear at the end of pregnancy. In a previous paper I have presented, with Pundel and Gandar, the history complete study of these modifications, so that for this symposium I should like to describe only the essentials.

After a systematic study of more than 4,800 smears from more than 700 patients at or near the end of pregnancy, it has been possible to establish several main criteria permitting the differentiation of three types of smears at the end of the pregnancy:

1. "Pregnancy prior to term" smear type,
2. "Pregnancy at term" smear type,
3. "Postpartum" smear type.

DESCRIPTION OF THE VAGINAL CYTOLOGY AT THE END OF PREGNANCY

1. "PREGNANCY PRIOR TO TERM" smear type

This smear type is identical with the normal pregnancy smear observed during the last two trimesters of pregnancy. It is composed of numerous clusters of intermediate cells, mostly of the navicular type. The Eosinophilic Index is below one, the Karyopyknotic Index (under phase contrast microscopy) is below 10. There is an absence of parabasal cells, mucus and red blood cells, and the leukocytes are rare. The vaginal flora is mostly composed of Döderlein bacilli in varying amounts.

2. "PREGNANCY AT TERM" smear type

In the last two weeks of pregnancy, the vaginal smear changes its pattern. The diminution of the number of cell clusters is the most important change and can be very rapid, occurring in one or two days. This disappearance of the cell clusters can occur to such a degree that typical clusters are found only in one out of every ten microscopic fields at low magnification. Furthermore, the individual clusters become smaller, but still consist of typical navicular cells. The cell vitality has not changed. We never could find any evident modification of the quality of the staining of the cytoplasm. Increases of the Eosinophilic Index between 2 and 15, generally between 5 and 6, and increases of the Karyopyknotic Index to 15 or 20 are the other main modifications shortly prior to term. Leukocytes remain absent or are few in number; no mucus, no red blood cells, and no parabasal cells are present. Döderlein bacilli are present, but may be of different types.

Summary: The "Pregnancy at Term" smear type is characterized by a marked diminution of cell clusters, the appearance of an increasing number of isolated and flattened cells and a rise of the Eosinophilic and Karyopyknotic Indices.

3. "POSTPARTUM" smear type in a pregnant patient at term

While in the "Pregnancy at Term" smear type the clusters of intermediate or navicular cells are still present in reduced amounts, these clusters disappear completely, so that nearly all cells appear single. Simultaneously, the Eosinopnilic and Karyopyknotic Indices continue rising, but in general the Eosinophilic Index rises to a higher level than the Karyopyknotic Index. The reason for the dissociation of the cytological indices is that a third cell type now appears in the smear, the parabasal cell, which, with a certain number of intermediate cells, rapidly takes the structure of the post-natal cells described by Papanicolaou. They have a round or oval form, with regular cellular outlines. Their cytoplasm can

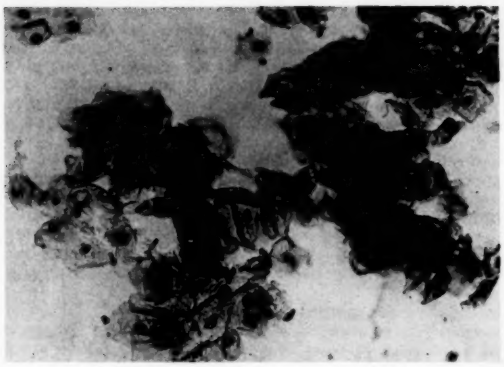

Fig. 1. "Pregnancy prior to term" smear type. Typical pregnancy smear. Smear taken at the 280th day of pregnancy. Spontaneous delivery 15 days later. Normal child.

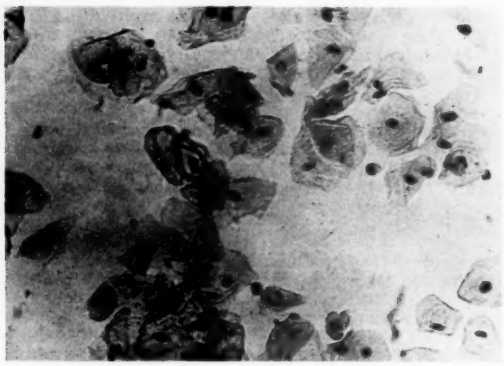

Fig. 2. "Pregnancy at term" smear type. Disappearance of the heavy cell clusters. Nearly all the cells are isolated: Only superficial or intermediate cells. Smear taken at the 280th day of pregnancy. Spontaneous delivery two days later. Normal child.

contain one or more vacuoles surrounding a large vesicular nucleus with a particularly fine and regular chromatin net. The diameter of the nucleus is larger than eight micra. A particular modification is that some parabasal and intermediate cells can show eosinophilic cytoplasm and their nuclei can undergo premature degenerative pyknosis. Leukocytes appear in increasing numbers. The occurrence of these parabasal cells indicates a marked regression of the hormonal activity of the placenta.

Summary: This smear type shows a progression toward the typical postpartum type, with disappearance of the navicular cell clusters and the appearance of parabasal cells of the postpartum type. By its loss of the typical cytological characteristics of pregnancy and the appearance of these new cell types, the smear can assume such patterns that it can no longer be distinguished from the typical postpartum smears after delivery.

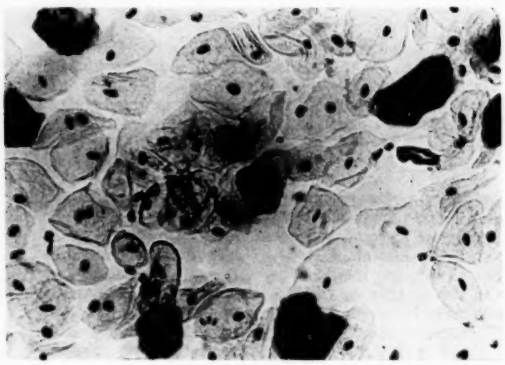

Fig. 3. Smear taken at the 295th day of pregnancy. The smear shows the "Pregnancy at term" type with a beginning transition toward the "Postpartum" type. Numerous eosinophilic superficial cells (in black), few navicular cells, appearance of some parabasel cells of the postpartum type. The following day Caesarean section had to be done because of severe fetal distress and transverse position. Baby 4,000 gm, 52 cm, but showing marked weakness in breathing the first two days, needing an oxygen incubator. Recovered.

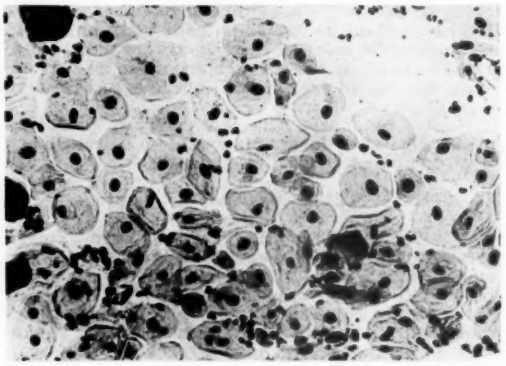

Fig. 4. Pregnancy 22 days past the expected term. Typical "Postpartum" type of smear. Numerous round or oval parabasal and intermediate cells with nuclear and cytoplasmic hypochromatism. Several parabasal cells are eosinophilic as seen in the postpartum smear during lactation. Immediate induction of labor, with success at the first trial by intravenous pitocin-glucose infusion. Spontaneous and easy delivery of a very weak baby weighing 2,700 gm, length of 54 cm. Typical post-mature baby with marked dehydration and maceration of the skin. In spite of oxygen treatment, the child died a few hours after birth.

LIMITS AND DIAGNOSTIC DIFFICULTIES OF THE VAGINAL SMEAR AT THE END OF PREGNANCY

1. A correct diagnosis can be done only if the entire smear is examined, at first under low magnification, then under higher magnification (400 x).

2. The smears must be taken exclusively from the lateral wall of the vaginal cul-de-sac, after insertion of a dry speculum. Contamination of the smear with cervical mucus or cervical cells must be strictly avoided.

3. The fetal membranes must be intact.

4. Infection and cytolysis can render a precise diagnosis impossible. In these cases the cellular patterns must be cleared up by local treatment (antibiotics: two vaginal tablets daily of oxytetracyclin for two days). The smear then is cleared up sufficiently at the third or fourth day after therapy.

Table I

SMEAR TYPE	PREGNANCY BEFORE TERM	PREGNANCY AT TERM	POST PARTUM OR OVERMATURITY
Cell clusters (navicular cells)	+++	+ or 0	0
Isolated, single cells	+	++ or +++	+++
Isolated superficial cells	0	++	+++
Isolated intermediate cells	+	++	+
Parabasal cells	0	0	++
Eosinophilic Index (+)	-1	2 - 15	10 - 20
Karyopyknotic Index (++)	-10	+ 10	+ 10
Leukocytes	(+)	(+)	++
Red blood cells	0	0	0 (+)
Mucus	0	0	0 (+)

THE DIFFERENT CYTOLOGICAL MODIFICATIONS IN THE VAGINAL SMEAR AT THE END OF PREGNANCY

(+) The Eosinophilic Index includes only the percentage of the eosinophilic superficial cells, with a completely pyknotic nucleus (diameter less than 6 micra).

(++) The Karyopyknotic Index includes only the percentage of the pyknotic nuclei of the superficial cells.

RESULTS

In a series of 713 pregnant patients the vaginal cytology was studied near the expected term of delivery, based upon standard calculations: 280, plus or minus ten days after the beginning of the last menstrual period. The comparison of the cytological findings with the clinical findings permitted the first general conclusion that neither the parity nor the anatomical condition of the cervix nor the prenatal engagement of the fetal head in primiparas have any influence upon the vaginal cytology, so that the cytological findings are identical and of the same significance in primiparas as in multiparas.

Of the above 713 patients, 315 showed the "Pregnancy Prior to Term" smear type (TABLE II). Only ten patients, or 3.12 per cent, delivered spontaneously within the first five days following the cytological examination. These exceptions are not significant and can not be considered as errors of the method, because vaginal cytology reflects only the hormonal activity of the placenta and because delivery may be induced in some cases by non-hormonal factors which can not be visualized in terms of changes of vaginal cytology. The causes of the delivery in these exceptions have been hydramnions, toxicosis, uterine tumors and multiple pregnancy.

TABLE II

"Pregnancy Prior to Term" Smear Type	315 cases	
Spontaneous Delivery Within 5 Days	10 cases	3.12%
Spontaneous Delivery After 5 Days	305 cases	96.88%

Three-hundred and ninety patients showed the "Pregnancy at Term" smear type and of these, 359 (or 92%) had spontaneous delivery within the following five days (TABLE III). A careful study of the remaining 31 patients (who did not deliver spontaenously during these five days) revealed that the uterus in these patients presented a certain degree of inertia for the normal start of the birth contractions or some disturbance of the normal mechanism of the induction of labor, and that they were potential cases for post-maturity.

In eight pregnant patients, the smear exhibited the typical "Postpartum" type. Artificial induction of labor was started immediately, but unfortunately, only four babies could be saved. The other four died shortly after delivery. All eight babies showed the classical signs of post-maturity in varying degrees, such as length over 54 cm, dehydration and marked maceration of the skin.

TABLE III

"Pregnancy at Term" Smear Type	390 cases	
Spontaneous Delivery within 5 Days	359 cases	92%
Spontaneous Delivery After 5 Days	31 cases	8%

The period shortly before term beginning with the occurrence of the "Pregnancy at Term" smear type indicates progressive lowering of the hormonal activity of the placenta, and is characterized by a progressive disappearance of the so-called vaginal inertia against estrogens. This biological phenomenon can be used as a test in cases where the vaginal cytology does not yet show the typical pattern of the "At Term" smear, but in which the obstetrician would like to know if the pregnancy is at term or not. If the pregnancy is really at term, the injection of 20 mg. of estradiol monobenzoate produces an increase an increase of the Eosinophilic and Karyopyknotic Indices, while in the cases where the pregnancy is not yet at term or where a premature delivery is unlikely, the estrogens produce no change in the vaginal cytology (18, 19).

CONCLUSIONS

At the end of the pregnancy, the vaginal cytology shows some special modifications permitting the individualization of three main types of vaginal smears and are characterized by progressive disappearance of the typical pregnancy cytology and gradual transition to postpartum cytology.

The "Pregnancy Prior to Term" smear type permits one to affirm with an accuracy of over 95 per cent that the delivery will not start within the next five days.

If the smear shows the "Pregnant at Term" type, spontaneous delivery will occur in the next five days in over 90%. The remaining cases have to be followed carefully, since they are potential cases for post-maturity.

If the smear shows the postpartum type, labor should be induced immediately, to avoid possible fetal distress by post-maturity.

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NILO PEREIRA LUZ

Porto Alegre, Brazil

Modifications have been described in vaginal cytology, mostly by Lemberg-Siegfried and Stamm (1). Previously some other authors (2) had observed similar changes, ascribed by them to the hormonal alterations that precede labor. Lemberg-Siegfried (1), Lichtfus (3) and others think that these alterations mean that pregnancy is approaching term. These changes are supposed to be present in most cases 15 days before normal labor starts. The authors claim that labor cannot be induced in a large percentage of cases, if these signs are absent at the time when induction is started.

What modifications have been described?

a. Increased number of superficial cells on the smear (the intermediate type of cell which is found commonly during pregnancy is observed together with variable amounts of large polygonal, flat cells with pyknotic or pre-pyknotic nuclei). Occasionally deep cells with unusual staining reactions are seen.

b. Loss of the normal clumping that predominates as the most usual way in which cells are shed in a pregnancy smear. Increased number of isolated superficial cells are found, mixed with abnormal amount of mucus (which incidentally is rarely seen in a normal pregnancy smear).

c. Abnormal tinctorial reactions: increased eosinophilic cytoplasmic discoloration (Lemberg-Siegfried).

d. Increased number of leukocytes, in absence of any vaginal infection or infestation. The leukocytes are seen most commonly mixed with cervical mucus but can be seen completely mixed with the vaginal cells.

Anyone studying vaginal smears during pregnancy has already found this picture. The important point that deserves to be scrutinized is the real frequence of its presence and its etiological cause, which means the real relation it bears to the age of pregnancy, hormonal conditions that precede labor, and how it is produced on the smear. On this matter, it seems to us that many theories have been advanced beyond the limits that could be expected from the given evidence of observed facts.

For instance, Davis and Pearl (4) were not able to demonstrate any differences between antepartum and immediate postpartum vaginal biopsies.

Caldeyro-Barcia and Alvarez (5) have shown how early in pregnancy the antepartum period starts and that the only detectable sign of progressing labor, in addition to regular uterine contractions, is the effacement and dilatation of the cervix. It seems to us more important to interprete the modifications of vaginal smears as the consequence of mechanical alterations caused by cervical effacement and not as a symptom of hormonal changes that should indicate the termination of pregnancy.

The following are my own observations:

1. We have consistently found the described alterations in the great majority of patients with severe threatened abortion. They are extremely frequent in patients that are in active premature labor or expulsing an nonviable fetus.

So we keep the impression that the described alterations "shortly prior to term" are related to impending or actual labor and not to term or duration of pregnancy.

2. We have observed occasional cases, after excessive physical activity or external trauma with a rising Karyopyknotic Index, with an increase of the number of superficial cells and in whom the clumping of the cells becomes extremely scant and the amount of leukocytes increases. Bed rest, sedation and estrogenic therapy restore the typical picture of a normal pregnancy smear, if it will go until term. These observations were found around the sixth and seventh month of pregnancy. Again we must consider

that these alterations (described as specific of a "term pregnancy") are a sign of impending interruption of the pregnancy. If one does not intend to interfere with normal fetal development and maturation, it seems to us that one should not assume that a pregnancy is "at term" only on the basis of vaginal cytology.

3. If care is taken to avoid contamination of the smear with cervical cells when preparing the vaginal smear, we could not observe any cellular change in normal pregnancies at term or even during labor if the fetal membranes were intact. We observed patients in actual labor in whom the cell patterns show no alterations at all. We must then consider that the vaginal smear modifications found near term are not necessarily an indication that normal labor will start and/or continue. They are not related to the cause or causes of labor in the same way as they are not related to the duration of pregnancy.

4. From our material we have the impression that the different staining reaction of cellular cytoplasm observed near term is the expression of a very slight degree of dehydration that occurs before fixation has been effective. This is caused by the fact that the isolated cell is very quickly dehydrated. It can be easily observed if one compares the staining reaction of the isolated or crowded cells on the same smear.

In conclusion we can say that more investigations are needed, if one intends to diagnose the normally terminating pregnancy by means of the vaginal smear.

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LUIS MONTALVO-RUIZ

Madrid, Spain

Vaginal cytology at the end of pregnancy is discussed first during the normal pregnancy at term and second during the prolonged pregnancy.

Normal pregnancy at term is defined as one that lasts for more than 39 weeks and less than 41 weeks. From 41 weeks onwards we consider it a prolonged pregnancy.

90 PREGNANT WOMEN EXAMINED

Normal pregnancy at term prior to onset of labor	32
Normal pregnancy at term during labor	46
Prolonged pregnancy	12
Total	90

RESULTS

In the group of normal term, pregnant women before the beginning of delivery, we observed 27 with normal smears of pregnancy, either navicular or cytolytic, with an Eosinophilic Index smaller than 5 and a Karyopyknotic Index under 10. The other five showed infected smears, with abundant leukocytes and coccoid bacteria which precluded hormonal evaluation.

Of 46 women in labor, 45 were in the first stage and one in the second stage. Eleven of these 46 pregnant women had infected smears and of the remaining 35, 28 showed normal smears of pregnancy, and seven showed disappearance of the navicular cells and some parabasal cells of the postpartum type.

In the group of 12 prolonged pregnancies we observed: eight smears of normal pregnancy; two infected ones and two with the cytologic modifications mentioned above. We carried out a hormonal test with the eight pregnant women of this group who had no alterations in the smears, injecting 20 mg. estradiol benzoate, and in six of them there was found an increase in the eosinophilia and the pyknosis. These eight women delivered their babies spontaneously within eight days after the test.

SUMMARY

Out of 72 pregnant women with "clean" vaginal smears of normal pregnancy we found 9 (27%) of them with cytologic modifications of the type described by Lemberg-Siegfried, Stamm and de Watteville (1), Lichtfus, Pundel and Gandar (2). In our observations, the percentage of alterations of the vaginal

smears varies considerably. We believe that our material is too limited to arrive at definite conclusions and believe that other hormonal tests must always be carried out to supplement the cytologic findings.

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J. PAUL PUNDEL
Luxembourg, Luxembourg

In the monograph I published in 1951 with Van Meensel, we concluded the following concerning the cytology at the end of pregnancy: "We have not observed any particular modification of the smear during the last two weeks of pregnancy, with the following possible exceptions: that in some cases the Karyopyknotic Index may rise moderately or that the vaginal cells may lose their vital picture to some degree and appear more opaque. But the problem merits further investigations."

We did not present more precise conclusions for the following reasons:
(1) Our material was not sufficient to permit statistical conclusions.
(2) It was not always possible to obtain smears in a correct manner, because the medical staff in the prenatal and obstetrical services was not yet sufficiently accustomed to the cytological technique.
(3) We would not present conclusions because it was theoretically possible that the observed modifications had a pure mechanical origin by the prenatal distension of the cervix or the prenatal engagement of the head, thus causing some distension of the upper vaginal part.

In 1955 when Lemberg-Siegfried and Stamm presented their interesting papers concerning the particular modifications of the smear before term, I asked my associate Dr. Lichtfus to again study the vaginal cytology at the end of pregnancy in order to determine if these modifications are of hormonal or mechanical origin. For this investigation it was necessary that the smears were taken only at the lateral wall of the vaginal cul-de-sac, and compared not only with the clinical findings, but also with smears taken from other parts of the vagina and the cervix.

With this investigative program and with sufficient material we could not only confirm the cytological findings of Lemberg-Siegfried and Stamm, but also conclude that the cellular changes are independent of mechanical factors and may bo of important practical value for the obstetrician.

I will not repeat the cytological descriptions of the smears at term which have been presented by Lichtfus in this symposium. I would only repeat that the typical modifications of the vaginal smear at the end of pregnancy are observed very easily, but only if the smear has been taken very carefully from the lateral part of the posterior vagina and not from another site. Smears taken without a speculum and without these precautions are generally useless.

As the cytological findings are being described by others in this symposium, I would like to speak now not as a cytologist, but as an obstetrician, and I would like to present the practical value of the vaginal smear for the obstetrician.

The correct diagnosis of normal pregnancy at term remains one of the most annoying problems for the obstetrician, because the fetal mortality has been proven to be very high in cases of true postmaturity, but on the other side, the induction of labor to avoid postmaturity can also be harmful for the infant. At present, the obstetrician has no objective clinical criterion for the exact diagnosis of postmaturity, as shown at the last Congress of the French-Speaking Societies of Obstetrics and Gynecology in Marseille in 1957. For the diagnosis that a pregnancy is at term or not yet or already over a term, the obstetrician generally can rely only on one piece of information: the date of the last menstrual period. This date supplied by the patient is not always correct. However, if a patient seems to be really ten days over term, the obstetrician is presented with a real problem which engages his responsibility and his professional skill. Should labor be induced or not? That is the question which is very difficult, and in some cases even impossible to answer without the aid of vaginal cytology.

If in a pregnancy at term, plus ten days, the obstetrician decides to induce labor, he can risk obtaining a premature baby or he can, if the first induction is ineffective, produce a complicated psychological reaction in the patient. He has started a treatment for some reason, and if he discontinues his efforts later, the patient may enter into a state of anxiety and ask if the first treatment has done harm to her baby. If the obstetrician repeats the induction, he takes the risk that in many cases he will have to repeat the treatment several times, and it is quite possible that such a treatment may have a harmful effect on the child if not on the mother. In my opinion, I still believe that at least a part of the high fetal mortality rate in cases of suspected postmaturity is not the result of the postmaturity, which exists only in rare cases, but of the forced induction of labor, especially if it is done by administration of massive dosages of quinine according to the Stein method.

If the obstetrician prefers to wait for spontaneous labor to start, he risks losing the child if it is a case of true postmaturity, in which case it may be followed by a claim for malpractice.

But as the date of the last menstrual period is a rather unreliable indication, since in some patients regular bleeding can continue during the first three months of pregnancy, there can exist true cases of postmaturity which would not be detected by the classical calculation of the term.

I have collected with Lichtfus rather extensive material (over 800 pregnancies at term), in which we have found existing a particularly high correlation between the modifications of the vaginal smear and the onset of spontaneous delivery. The incongruences are not higher than eight percent, but even these are not errors due to the method. They are due to the fact that also other non-hormonal factors can influence the onset of labor, such as hydramnions, uterine tumors or malformations, toxicoses, etc.

In order to show the practical value of the vaginal smear at the end of pregnancy, I have selected 157 consecutive cases from the material that I have studied with Lichtfus, where vaginal smears were requested by the obstetrician, because these patients presented a problem for the correct diagnosis of the term. They can be divided into two groups:

I. Patients who were beyond their calculated term for more than ten days. In 58 of this first group of 103 patients, the vaginal smear was of the type "Pregnancy Prior to Term." Spontaneous delivery followed only later, when the smear changed to the type "Pregnancy at Term." In one patient this happened at the 328th day of pregnancy as calculated from the last menstruation and there was no evidence of postmaturity. There were three exceptions: one patient had Cesarean section for serious bleeding due to placenta praevia, and in two other patients, labor was induced because spontaneous delivery did not occur within five days after the smear had changed to the type "At Term."

In 36 patients the smear exhibited the type "Pregnancy at Term." Of these, 24 delivered spontaneously within the next five days. In the remaining 12 patients, labor was induced later, as spontaneous delivery did not occur within five days. In one patient, the smear changed toward the "Post-Partum" type within four days. The infant in this case was the one who showed fetal distress even before induction of labor, but all 12 infants were alive. The induction of labor by intravenous ocytocic infusion had success at the first trial in all 12 cases.

In nine patients the smear exhibited the "Postpartum" type. In spite of immediate induction of labor, four babies died in the first hours after delivery from extreme weakness of respiration, in spite of oxygen treatment. All nine babies showed the typical clinical picture of post-maturity (length over 54 cm., marked dehydration and maceration of the skin).

The vaginal cytology in this first group permitted avoidance of unnecessary induction of labor in 79 cases where the obstetrician could safely wait for spontaneous delivery as long as the vaginal smear was not alarming. It permitted, therefore, one to limit artificial induction of labor to only 23 cases, with the loss of four babies. However, I am convinced that these four babies could have possibly been saved if the pregnancies had been followed earlier with repeated smears, and if labor had been induced at the right moment as determined with cytology.

II. Patients with doubtful duration of pregnancy. Either the last menstrual period could no longer be remembered by the patient or there existed clinically an incongruence between the size of the uterus (with one child) and the time period which allegedly elapsed since the last menstruation. Twenty-four of this second group of 54 patients showed the smear type "Pregnancy Prior to Term." None had spontaneous delivery before at least ten days. In 28 patients the smear exhibited the "At Term" type. Spontaneous delivery occurred in all cases within the next five days. In two patients the smear showed the "Post-Partum" type. Immediate induction of labor was started and resulted in the delivery of a living but weak infant in both cases showing the classical signs of postmaturity.

These results have been and continue to be very demonstrative. I have accepted for my obstetrical practice the following conclusions which are observed now systematically in the Obstetrical Department of the Maternite Charlotte in Luxembourg (annual delivery rate of 1700):

1. Vaginal smears are taken at weekly intervals from every pregnant patient, at least after the beginning of the ninth month.
2. If in a case of suspected postmaturity the vaginal smear remains of the "Pregnancy Prior to Term" type, no induction of labor should be done as long as this type persists.
3. If there exists no other serious obstetrical indication, induction of labor for convenience of the patient should be done only if the vaginal smear shows the "At Term" type.
4. If a patient shows the "Postpartum" type and the fetus is still alive, induction of labor should be done immediately, even if the patient seems to be prior to term. As in these cases, the induction of labor has nearly 100 per cent immediate success; it can be prepared by rupture of the membranes without any risk for mother or child.
5. If a patient presents signs of threatening premature delivery, as calculated from the last menstrual period, a vaginal smear is taken. If it shows the "At Term" smear type which can exist also in cases of threatening abortion, the administration of estrogens will show if the premature delivery can be avoided or not. If the vaginal smear shows a typical estrogenic response, delivery will occur and in general it is a delivery of a baby at term. If the vaginal smear shows a return to the normal pregnancy smear "Prior to Term," chances are that it was only a threatening of premature labor, and that delivery will not occur within the next two weeks.

These lines of obstetrical conduct at the end of pregnancy have had as a result a statistical valuable improvement of fetal mortality for the following reasons:

1. One could discover cases with imminent prenatal fetal death where the child could be saved by immediate induction of labor or Caesarean section.

2. One could avoid numerous unnecessary inductions of labor, even in patients who had lost one, two and even three children during previous forced inductions of labor because the pregnancy seemed to be past term. Due to the changes observed in the vaginal smear we have been led to await the spontaneous delivery of a living infant, and this had occurred in some cases after the 310th day of pregnancy.

3. The vaginal smear permits one to find out with a maximum of accuracy if a pregnancy is at term or over term, regardless of the last menstrual period. It permits one to conclude that true postmaturity is a complication of pregnancy which occurs only rarely and that over 80 per cent of the suspected cases of postmaturity are normal pregnancies in which the last menstrual period was incorrectly remembered or ovulation did not occur at the usual time or in which the child needed a longer than normal time for his complete prenatal development.

The vaginal smear in pregnancy is not a universal test permitting the detection of every possible complication of pregnancy and delivery. Vaginal cytology deals only with the hormonal function of the placenta and other causes of premature labor or fetal distress and death may exist, such as toxemia, hydramnion, erythroblastosis, placenta praevia, uterine tumor, etc., the existence of which can not be detected or evaluated by vaginal cytology. However, if used within the limits of its biological purpose and harmoniously integrated in the usual obstetrical investigations, one can honestly say that vaginal cytology merits systematic use in the obstetrical practice, not only for the control of the pregnancy and treatments for threatening abortions, but also for defining the time when the pregnancy can be considered as being at term. In my opinion and experience, the vaginal smear is, at the moment, the only test which permits one, without any risk for the patient and in a very easy manner, to find out if a pregnancy is at term or not. The accuracy of the vaginal smear is without any doubt much higher than that of other tests such as hormonal assays, ocytocic tests or repeated X-rays, investigations which are not possible to use in small hospitals or are not always without risk for mother or child.

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For complete references, see the bibliography after the paper by Lichtfus in this symposium.

DISCUSSION

FRANTIŠEK HORÁLEK and MOJMÍR SONEK, Brno, Czechslovakia:

In a thorough discussion of this subject we would be obliged to repeat what we have already said in other discussions in this issue. That is why we shall limit ourselves to a few comments.

We are surprised by the high percentage of failures in the cytological diagnosis of the pregnancy at term, as stated by Montalvo-Ruiz. We, on the other hand, had very good results with this method. A certain small percentage of diagnostic failures can be explained by an insufficient estrogenic predominance in hormonally unprepared labors. This exception is also mentioned by Lichtfus, and on the basis of our research some cases of labor complicated by a premature rupture of the membranes and some cases of protracted labor with primarily weak uterine contractions could be added.

Some difficulties can be encountered in cases with vaginal infection. Here, a great deal of help is provided by simultaneous intravaginal application of antibiotics and parenteral injection of estrogens. Our method is similar to Lichtfus' with the only exception that in uncertain cases we combine both treatments. Most often this combined treatment completely clears the cytological picture.

The problem of the smears of prolonged pregnancy remains to be solved. We found these smears in some deliveries where healthy babies were born without any signs of post-maturity. We have not yet found a precise explanation for this discrepancy.

MARIO DE BENNING KAMNITZER, Rio de Janeiro, Brazil:

In 1952 O. Rodriques Lima and I demonstrated the colpocytologic changes which precede the clinical onset of abortion and full term labor.

In 1953 I described the decrease of proliferation of the vaginal epithelium during the ninth month of pregnancy and the sudden smear changes which take place some time before clinical onset of labor (1). I estimated this time to be from 24 to 48 hours before labor and pointed out that premature labor and abortion may be preceded by similar changes, adding, however, that in cases of prenatal death of the fetus the aspect is different.

The vaginal smears used for this study were in most cases obtained from patients with living babies who had symptoms of impending labor, such as painful contractions, pelvic pains or slight mucous spotting, but presented a long or just "ripe" cervix and a definitely closed internal os. Cervical dilatation and effacement began, in these cases, in a matter of a few to 48 hours.

All patients (50), except two cases of "false" labor, which went into real labor a week later, exhibited striking smear changes as follows:

About 24 to 48 hours before the clinical onset of labor there appears a radical modification of the smear. The "agglutination phenomenon" of the cells disappears and the leukocytes become more numerous. Flat cells with distended cytoplasm and pyknotic nuclei frequently appear. The eosinophilia of the smear is high, especially noticeable in the intermediate cells. The vaginal flora is changed. One observes a new proliferation peak with a Karyopyknotic Index of about 30 (1).

As soon as labor begins, one observes a rapid decrease of the Karyopknotic Index; meanwhile the Eosinophilic Index rises, for a short period reaching values sometimes over 60. Later the eosinophilia decreases and after expulsion of the fetus, it remains around 40%. The vaginal smear observed in the period preceding labor is also characterized by the presence of some external basal cells, cervical cells and round, large intermediate cells (1).

The examination of successive smears made during labor reveals a decrease of the number of superficial karyopyknotic cells and the appearance of rounded intermediate cells with large nuclei and a doubtful chromophilia. These cells are sometimes cyanophilic, sometimes eosinophilic and sometimes they do not stain at all. Occasionally the cervical cells are very abundant, being mostly eosinophilic. On the other hand, the basal cells become more frequent. The cytological pattern gives the impression of an intensive desquamation of the superficial layers of the epithelium with a contemporary cessation of proliferation, as indicated by the appearance of basal cells (1).

These findings were repeatedly published since 1953 (2, 3, 4, 5, 6, 7) but they have remained unconfirmed for nearly two years and their existence was on several occasions denied. I began, therefore, to feel quite distressed, but fortunately in 1955 the important paper of Lemberg-Siegfried and Stamm (8) appeared.

Having read the paper of Lemberg and Stamm (8) I recognized in their photographs some very familiar colpocytologic pictures which I have seen either two weeks before delivery (pregnancy prior to term) or 24 to 48 hours before delivery (pregnancy at term). Similar pictures were found in 1957 by Lichtfus and co-workers (9). In my monograph (1) I printed a photograph of the cytological pattern actually called "pregnancy prior to term". But I have seen this same pattern in other circumstances in pregnancy smears not related to term, and I discarded it as an unreliable sign. I regard it, however, as an early sign of the genital crisis of the vagina which seems to begin before the clinical onset of labor, be it a full term pregnancy or not. In this matter I agree entirely with Luz.

Furthermore, our experience has shown us that the genital crisis during puerperium, as revealed by vaginal smears, is initiated either by the prenatal death of the fetus (in this circumstance the appearance of the regressive changes is subject to wide individual variations) or at the appearance of peculiar uterine contractions, with a pattern completely different from that of the normal pregnancy contractions, the only ones capable of producing dilatation of the cervix and expulsion of the fetus.

Caldeyro and Alvarez (10), in their work on uterine contraction, have extensively shown how the uterine contractility of pregnancy changes and "matures" to the genuine labor pattern. They also explain how this "maturing" process, which may be induced or accelerated by many agents, usually develops very slowly near term and that it can take days before the definite change to the labor pattern is completed.

From this it can clearly be seen that the smear changes which I and others have described as just "prior to labor" occur simultaneously with the above mentioned changes of uterine contractility (1).

This leads to the heart of a conflict of opinion:

I will try to scrutinize the two issues which represent the focal point of the differences. The first is of a purely theroretical nature and the second concerns the collection technique.

1) The concept "term," as used in obstetrics, has a chronological and a qualitative meaning. It implies that pregnancy has reached the so-called normal duration and that the baby is most probably mature. The generally accepted average for the duration of pregnancy ranges from between 280 and 282 days post-menstruation, and Hosemann (11), in his statistical studies, concluded that the normal duration of pregnancy is limited to a period between the 267th and the 296th day after the last menstrual period in women with normal, 28-day cycles, and that this period includes 80% of the labors resulting in mature babies.

"Term," therefore, is a clinical and statistic concept which can eventually only be confirmed by the occurrence of labor and the examination of the newborn.

Colpocytology cannot tell us if the duration of pregnancy has reached the normal range, and still less if the baby is mature, but it does tell us - sometimes with astonishing accuracy - whether or not labor is at hand, even before clinical symptoms occur.

This is also the reason why colpocytology is so successful in determing the most favorable moment for induction of labor, because labor is already on the way.

If onset of labor, as foretold by colpocytology, is really at term, before term or after term, is, in my opinion, another question, more related to obstetrical practice than to the microscopical examination of vaginal smears.

There is another important concept which deserves discussion because it touches a most controversial subject. Pundel says that vaginal cytology deals only with the hormonal function of the placenta and that other causes may influence the onset of labor. Lichtfus takes the same point of view: "because vaginal cytology reflects only the hormonal activity of the placenta, and because delivery may be induced in some cases by non-hormonal factors which cannot be visualized in terms of changes of vaginal cytology."

Are there really some labors induced by hormonal and others by non-hormonal factors? What hormones do the main speakers have in mind in order to explain such a duality of causes?

I doubt that vaginal cytology during the premonitory period preceding labor reflects only the hormonal activity of the placenta. If one observes the structual modifications of the subepithelial tissues of the vaginal (and cervical) epithelium which appear in early labor, even before dilatation of the cervix takes place, one does not wonder that the vaginal smear goes crazy.

I have observed that estrogens may prevent the appearance of the slight regressive cytologic changes in "prior to term" smears, but I have never noticed any such influence on the colpocytologic pattern of the "antepartum" smear.

2) Pundel stresses the importance of his collection technique for an accurate cytohormal diagnosis, and I agree that he is right for it happened to me one time that in a case of suspected premature labor I collected two smears (at the same time) with conflicting colpocytological patterns. One smear was a normal pregnancy smear, but the other exhibited the peculiar pattern which precedes labor. However, the patient went into premature labor within a period of three days. I began to wonder and decided that this strange finding must have something to do with my collection technique.

I used to collect smears during the pelvic examinations as follows: The vulva is rinsed and cleansed with wet gauze. The bi-digital vaginal examination is performed as usual. After withdrawal of the fingers a clean slide is passed over the tip of the middle finger and a second slide is passed over the secretions accumulated between the middle finger and the index finger. The smears are fixed immediately in alcohol-ether, and finally stained by the Papanicolaou technique.

Later I started collecting smears with the use of a speculum from the cervix and from a lateral wall of the posterior part of the vagina, on patients during the suspected period immediately preceding labor, but with a closed cervix. I took the utmost care to avoid contamination of the spatula with other parts of the genitals.

I examined 23 cases in this latter way, but I have never again seen such a startling dissociation of colpocytological patterns as mentioned above. However, in nine cases a well recognizable difference could be found concerning the eosinopholic, karyopyknotic superficial cells, which appeared in a much higher percentage in the cervical smears. Also the rounded intermediate cells of varying chromophilia and the parabasal cells, if present, were definitely more numerous in the cervical smears. In 14 cases, which incidentally went into premature labor, no clear difference could be found.

Pundel says that smears which are not taken from a lateral wall of the posterior vagina and without the use of a speculum are useless.

But then why do patients exhibiting the above mentioned type of smear, collected during the vaginal examination, go promptly into labor in a matter of a few hours or a few days? The prognostic accuracy of the above obtained colpocytological pattern is undeniable, and I have found it also in cases of abortion and premature labor often before the appearance of clinical symptoms.

I do not have much experience with the cytology of prolonged pregnancy, because this entity seems to be rare in Rio de Janeiro. At the University Clinic we do not, as a rule, induce labor, for we believe that prolonged pregnancy is, in most incidences, a creation in the mind of nervous obstetricians.

As a matter of fact, we do not fear prolonged pregnancy, but we do fear prolonged labor, and we fear most the fetal hypoxia resulting from uterine hypertonicity, a condition most likely to occur when oxytocic drugs are used for induction of labor.

The main obstetrical problem in fetal postmaturity lies in the transition from intrauterine to extrauterine life, where fetal mortality is terribly high, as the figures presented by Pundel and Lichtfus show; for as long as a postmature baby remains in a resting uterus it is less endangered than in labor. This is a most annoying situation which seems to call for a caesarian section, not for induction of labor.

However, the prenatal diagnosis of fetal postmaturity is very difficult, because prolonged pregnancy does not necessarily imply post-maturity (but) the reciprocal is certainly not true. Therefore, the obstetrical problem of postmaturity presents itself only after a pregnancy has already become prolongated. There seems no possiblity of prophylaxis for this condition.

Colpocytology alone, or aided by the estrogen test, may inform one at a given moment whether the onset of labor might be expected in a time ranging from a few hours to a few days. This is small help for the problem under discussion.

Lichtfus, however, has shown the adverse meaning of the appearance of a postpartum colpocytological pattern in cases of prolongated pregnancy. In eight cases he induced labor with a result of four

dying babies. It was remarkable that all of them exhibited signs of postmaturity. Pundel and Lichtfus go so far in their argumentation that this colpocytological pattern reflects a great and immediate danger for the fetus that they propose, if the baby is alive, that labor should be induced at once, even if the pregnancy seems to be prior to term.

I have occasionally observed postpartum smear types at different stages of labor with living babies with no ausculatory signs of fetal distress. However, I do not think that this observation might be compared with the cases of Lichtfus, since all circumstances and especially the collection technique were different.

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OTAKAR NYKLÍČEK, Náchod, Czechoslovakia:

I quite agree with the statements of Lichtfus and Pundel (1). The dynamics of vaginal smears the week prior to delivery and the ten days following it, as reported, also conform with the views of both these authors.

We do our evaluation under the same conditions as Lichtfus recommends and use quantitative evaluation by means of our vaginal cytogram.

As far as the nomenclature is concerned, I suggest for discussion a change of the term "Postpartum smear type." I recommend the name "Pregnancy Regression" type. From the philological point of view "Postpartum" is a time which is already after delivery, whereas the cytological type which we describe, (that is, the appearance of parabasal cells) is seen during pregnancy, that is antepartum.

In order to express by an abbreviation the cytological characteristics of dynamic changes of vaginal smears, we use the terms "the shift to the right" and "the shift to the left" for all phases of gynecological endocrinology as studied by means of cytology. We signify by the term "the shift to the right" in the cytology of pregnancy the transition from the type "pregnancy prior to term" in the direction of the type "pregnancy at term," which means an accumulation of cells from the upper layers of the vaginal epithelium (and in the vaginal cytogram the number of cells shifts into the columns, situated at the right).

On the contrary, after delivery or in "pregnancy regression" type, we speak about the "shift to the left," that is, cells from the lower layers of the vaginal epithelium (the number of cells shift into the columns situated to the left in the vaginal cytogram).

Vaginal cytology has become most valuable for the determination of the stage of pregnancy, e.g., if the woman is already at term, which has been calculated according to Naegele. It is only due to vaginal cytology that we have decreased the number of inductions to a minimum and that in the last 3000 deliveries no fetal death could be attributed to post-maturity.

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GUSTAVE RIOTTON, OTTO STAMM and V. RAWYLER, Geneva, Switzerland:

To Pundel: we are completely in agreement with the excellent paper by Pundel based on carefully studied material and with the conclusions full of common sense.

To Lichtfus: we sould like to make a few remarks concerning the criteria discussed by Lichtfus. We use a somewhat different terminology. The author's "prior to term" smear corresponds to our "advanced pregnancy" smear. In fact we see this type of smear from the fourth month on. In addition, we have a "near to term" smear characterized by a decrease in the clustering of navicular cells, appearance

of isolated cells and mucus and a slight increase in superficial cells and leukocytes. The Eosinophilic and Karyopyknotic Indices are not altered.

Moreover, we have two types of "pregnancy at term" smear instead of one. The one we see most often is characterized by isolated navicular cells (there are very few clusters), disappearance of cytolysis and increase of superficial cells. In addition there are clusters of leukocytes and abundant mucus, but the Eosinophilic Index is below six and the Karyopyknotic Index is less than ten. The second type of "pregnancy at term" smear is the one described by Pundel. That we have two types is probably due to the fact that, for clinical reasons, we cannot take the specimens with the aid of a speculum and must, therefore, use a pipette. We must add that, in spite of the inconvenience of the method, particularly in reading the smears, we have valid correlations between smears and clinical findings. This is particularly true for the course of induced labor. Contrary to Lichtfus we find some isolated superficial cells in the smears of "advanced pregnancy." However, in Table I the author mentions a Karyopyknotic Index less than ten. Since he considers only the superficial cells to have a karyopyknotic nucleus, it follows that these smears contain some superficial cells. Finally, in the "postpartum" smear he states that the Eosinophilic Index increases more than the Karyopyknotic Index because of the presence of eosinophilic parabasal cells, although he claims to count only superficial cells as eosinophilic ones. But these are minute details.

To Luz: We reply that the modifications seen near term are the same as those seen during abortion or threatened abortion. There is here a question of terminology. We always say: type "at term" for instance. It is very evident that such a smear appearing in the fifth month of pregnancy, for example, cannot indicate the imminent birth of a mature infant. These changes are only the indication for threatened abortion or abortion. We have never pretended that the smear could indicate the duration of the pregnancy (fifth or ninth month for example) but that it is related to the sequence of events (of which we do not completely understand the mechanism) occurring during delivery or abortion. It is for this reason that smears are so useful for the clinician. We have already described all of these aspects. For the clinical side of pregnancy smears we refer the author to the above excellent paper by Pundel and for the theoretical side to our paper in this edition. Besides, we do not agree with Luz that the modifications are the "consequence of mechanical alterations caused by cervical effacement," because we see the same modifications during a threatened abortion or we can induce them experimentally by lowering the estrogen stimulation in castrated women. In these two cases, nothing similar to cervical effacement is taking place. The cases described by Luz, which after external tramua are cured by the classical treatment for threatened abortion are, in fact, cases of threatened abortion and the change of the smear type is typical. It is strange that Luz has never been able to see the typical changes when he did not have contamination by cervical material. Here again we refer him to Pundel's paper. Pundel takes all of his specimens from the lateral wall with a spatula, avoiding cervical contamination and with this method he finds all the changes. As for the author's statement that the difference in color of the cells in smears taken near term must be due to dehydration as a consequence of delayed fixation, we will not discuss!

GUILLERMO TERZANO and JOSÉ MARIA MEZZADRA, Buenos Aires, Argentina:

It is generally agreed that a high Eosinophilic Index and a high Karyopyknotic Index with isolated cells instead of clumping and clusters, are reliable criteria for the vaginal smears of the so-called "pregnancy prior to term." (It is in some respects similar to the smears in cases of threatened abortion.)

In serial vaginal smears obtained during the last month of a normal pregnancy, it is relatively easy to find these changes in smears, and when this type of smear appears, delivery may be predicted within the next three to six days.

Parabasal cells have seldom been found in our cases. We base our prognosis on the pattern of the smear.

CLOSING REMARKS

NILO PEREIRA LUZ:

To Terzano: I quite agree with your implications on pregnancy prior to term: "It is in some aspects similar to the smear in cases of threatened abortion."

I could not entirely agree with your notion of "high Eosinophilic and high Karyopyknotic Indices." The difference could be a matter of staining technique. With the differential complete Shorr stain that I have used for some years, such high indices are not seen. There is, indeed, an increase in cornification, which is under 5 to 10% in most cases; the Karyopyknotic Index is substantially increased only in cases with previous progesterone deficiency.

To Horálek and Sonek: I agree that a thorough discussion of the subject would take us into unnecessary repetition. It is because of this, that I will undertake just two points:

1. The relationship of the observed cytological facts with the age of pregnancy would clear up if we could consider them as pathognomonic of either a "mature" pregnancy or only as a sign of a pregnancy about

to be interrupted at any age. The latter seems to be the more generally accepted idea, from the reports and comments.

2. I would like to promote comments on the physiopathology of the observed facts. To us the published reports do not give sufficient evidence of why and how cytological changes occur. I will more extensively comment on this in answering Kamnitzer's inquiries.

Dealing with your paper, I would not be very surprised by the finding of a relatively high percentage of failures on the diagnosis of delivery term. If one does cytologic studies on every patient that appears for consultation, he will have many more diagnostic failures than if one does cytology only on doubtful or special cases. Here, as in other investigative activities, the method of study is of paramount importance. The technique of collecting the smear can also greatly influence the results, as can the personal approach to the matter.

"No comment" on your judicious appraisal on infection. Most of us would agree with your approach.

Your comments on the smears of prolonged pregnancy are interesting. All of us have seen these pictures with term delivery of healthy babies. Since 1954, I have been quite engaged with this problem and I have consistently found that these cases demonstrate estrogenic insufficiency and/or excessive aging of the placenta. Histological studies show increased hyalinization of villi, with enlargment of the intima of the arterioles and indirect evidence of impaired circulation of fetal blood. We consider these cases as circulatory insufficiency or inadequacy of the placenta, and we take great care of the baby during labor and delivery. I should add that this picture can be seen before term and is usually associated with levels of estrogenic secretion of less than 50% of the normal expected excretion. In fact, we have assumed that a particular aspect of this type of smear is pathognomonic of estrognic insufficiency, and we have described in detail the cytological picture (1).

To Nyklíček: I would like to stress the importance of doing quantitative cytograms (which I call colpocytogram) on evaluation of pregnancy smears. Of course I agree with you that a "post partum smear type" is a very unfortunate name for a cytological picture which is observed ante partum. It could be described very easily by calling it hypotrophic, a name which is correct and descriptive, as it is related to the degree of proliferation attained by the vaginal epithelium. Your suggestion of "shift to the left" as related to the opposite picture of "shift to the right" is very adequate on a personal basis, but for generalized use it implies a not easily arrived at agreement on what side to put proliferation or atrophy.

On your final comment, I do not quite understand how cytology "has become most valuable for the determination of the stage of pregnancy, if the woman is already at term, which has been calculated according to Naegele." If I know that a patient is at term, I do not agree that cytology could be most valuable to determine that. Perhaps you wish to say that cytology is invaluable to appreciate the condition or state of pregnancy; this I could not applaud enough. It seems to me a complete redundance after having a typical menstrual and obstetrical story, to look for cytological signs of "term pregnancy."

To Kamnitzer: I must again stress the fact that it is hard to compare results when we do not use similar methods. Just for exemplification, I would mention your finding of a smear containing 30% karyopyknotic cells and from 40 to 60% eosinophilic cells. With my standards such a smear would be considered useless for cytological evaluation because of deep dessication with the differential stain of Shorr. Perhaps with the alcoholic basis of the Papanicolaou modified technique that you use, things are different.

I was very sorry to hear from your comments that Pundel considers as valueless, collection techniques different from his own. My personal and limited experience has shown that vaginal aspiration as used by Papanicolaou, Shorr, de Allende and others, remains the leading technique as far as hormonal studies are concerned. Concerning pregnancy studies, I began with Kamnitzer, followed with Pundel's method and for the past four years I have been aspirating from the lateral fornices with a vaginal pipette and strong rubber bulb. In no other way have I been able to see such slight differences in grouping or isolation of cells or to have such comparable smears for serial study.

I was very glad to hear from Kamnitzer that he entirely agrees with me that the cytological picture we are dealing with is specific for imminent interruption of pregnancy, should it happen at term or not.

Now let us go to the very heart of the problem:

Are there modifications on the vaginal epithelium (of hormonal nature) or are the described changes just the result of the mechanical interference of an effacing cervix?

To admit the first hypothesis, we should admit that prior to labor there is a transient and sudden change in vaginal reactivity to estrogens that would bring about an increased cornification; and we also admit that such a change is of such a short duration that it disappears as soon as the fetus and placenta are expelled. It is well-known that the vaginal epithelium remains irresponsive to parenteral or oral estrogens during the full period of puerperium and this fact is fully demonstrated by all experienced cytologists. As previously commented on in this issue, this kind of lack of responsiveness should be attributed to a modification in estrogen metabolism and not to the presence of circulating levels of progesterone or the presence of the placenta. This could happen, but it is not probable. Along the same line of thinking, we can consider the fact that for some authors, the changes are the result of a withdrawal of normal estrogenic action; for others it would mean the cytological picture of an increased estrogenic action. For the latter no explanation is given for the frequent and concomitant appearance of deep cells, mostly of the parabasal type.

And last but not least, if such a "hormonal preparation" for labor would be physiologic, it should have been present in all cases, or labor would not start, a condition which is not seen. Anyone with reasonable experience with pregnancy cytology has dealt with patients that entered labor with various kinds of smears.

As for the second hypothesis, if difficult to demonstrate, at least it is easy to understand on a logical basis.

Pregnancy brings about a strong epithelization of any endocervical tissue that is exposed outside of the cervical canal. The picture is typical to anyone who has done colposcopy examinations during pregnancy, and this excessive proliferation of epithelial buds is used to suggest the presence of pregnancy. Mild infections in these crypts are also a common finding. Estrogens concentrate in inflammed cervical or vaginal cells, as compared with cells in their normal environmental conditions; thus, they attain higher levels of estrogen impregnation than normal vaginal cells. It is quite comprehensible that when cervical ripening begins to appear (which begins earlier in pregnancy than is generally assumed), the mechanical modifications cause a stronger desquamation of these more superficial cells, together with parabasal cells of the squamo-columnar junction. Those cells that are shed into the vagina can be deposited on the vaginal walls and would be collected with any technique of obtaining material for cytology. The changes in chromophilia are due to two factors: partial dessication because lone cells dessicate more easily than crowded cells and impregnation with cervical mucus which can change the staining reaction of the cytoplasm. The same thing happens when one is studying serial vaginal smears to ascertain ovarian function. Near ovulation, since vaginal material is scant, it is not easy to obtain uniform staining of all vaginal superficial noneosinophilic cells, no matter how fast one is in collecting and immediately fixing the smear. Dessication in the first mild degree affects only the cyanophilic cells and only when exaggerated to a greater degree is the gross dominance of eosinophilia.

Even if difficult to prove and not as yet demonstrated, this idea is at least not in conflict with our classical knowledge of estrogen effect and metabolism in pregnancy.

On the problem of the post-partum type smear I have expressed my thinking when commenting on Nyklicek's criticism. I was very glad to hear that Lichtfus and Pundel corroborate the existence of a placental insufficiency which is unfavorable to the fetus. And I am very glad, not shocked, with the fact that cytology could bring us new signs of fetal distress in utero.

To Riotton, Stamm and Rawyler: If one would expect an answer, one should formulate criticism in a suitable manner, not directed toward the individual but directed at the report. On that level I would be very glad to answer every inquiry.

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J. PAUL PUNDEL and CAMILLE LICHTFUS:

It is very interesting to follow the discussions of the main papers concerning vaginal cytology near term. As it is a recent part of vaginal cytology, it is evident that some points need further discussion and research, but we are glad to see that there even now exists a great uniformity of findings and of clinical results from several clinics who have had occasion to study the cytology at the end of pregnancy for sufficient time and with sufficient material.

We agree completely with Terzano, Mezzadra, Horálek, Sonek, Nyklíček, Riotton, Stamm and Rawyler.

To Terzano and Mezzadra: We suppose that their statment "pregnancy before term" is a typing error for "pregnancy at term" or that the authors mean the smear type just before the onset of labor. If this is true, we agree completely with the discussants and their interpretation of this smear type, which is indeed similar to the cytological pictures observed in cases of threatening abortion.

To Nyklíček: We are grateful for his pertient comments concerning the terminology we have used. In a paper which will be published within the next weeks, we have already made the same comments, showing that cytology at term only presents the same dynamic changes reflecting the results of the estrogen-progesterone balance and the different degress of vaginal sensitivity for estrogens as during the rest of the pregnancy and nothing more. So it would be more logical to accept the terminology proposed by Nyklíček for the following reasons: The vaginal smear does not directly indicate that a pregnancy is at term or past term. It only reflects the hormonal status of the ovaries or the placenta and the particular vaginal sensitivity to estrogens. From these vaginal reactions correlated with clinical findings one can make indirect conclusions concerning the term or post-maturity of the fetus. Indeed, the "postpartum" smear type can be observed (1) after delivery, (2) in cases of true postmaturity before delivery and (3) in cases of existing or impending serious fetal distress before the clinical term of the pregnancy. Since in all of these three cases this smear type indicates exclusively an important regression of the hormonal activity of the placenta, it would be more logical to accept the term "pregnancy regression type" for all "postpartum" type smears observed before delivery, as this terminology would indicate more precisely the real biological processes responsible for these changes, which depend exclusively on the hormonal activity of the placenta and not the duration of the pregnancy. If this smear type is found in pregnancies, which after the usual calculations are supposed to be past term, it corresponds in general

with postmaturity. But this conclusion is not obligatory, since this smear type can also be found in pregnancies where the babies are just at term or even premature, if the placenta has undergone serious regressive changes.

There remains the terminology of the smear type "pregnancy at term" showing, as so well demonstrated by Nyklíček, a cytological shift to the right. We think that this terminology "pregnancy at term" can be preserved, as this smear type reflects a particular and rather specific hormonal reaction. It can be similar to smears observed in cases of threatening abortion, but a normal estrogenic response, as in nonpregnant women after a test treatment with estrogens, which characterizes the smear type "at term" shows in practically all of these cases that the pregnancy has arrived at its biological term, which is not identical with the calculated term. Therefore, this terminology seems to be correct, even in pregnancies before the calculated term, if one would accept this terminology as meaning the biological term or end of pregnancy compared with the calculated usual term and it can arrive before as well as after the calculated term.

To Horálek and Sonek: The possibility of the appearance of the "regressive smear type" outside of true overmaturity has been explained in our reply to Nyklíček. We are glad that these discussants could obtain healthy babies in such cases, a chance we had only rarely in our material. But this difference could be a question of the time elapsing between the first appearance of this smear type and the moment of delivery. We also believe that if this smear exists only for a short time the baby can be born in a rather satisfying condition of health, but in general, this smear type is associated with intrauterine hypoxia of the fetus and the fetal mortality is directly proportional to the number of days during which this smear type existed before delivery.

To Riotton, Stamm and Rawyler: We have been particularly interested in the comments of these discussants because they are the promotors of the use of the vaginal smear at the end of pregnancy. There exist some differences between their terminology and our own, but we also believe that they are only small details of personal convenience and are not the result of theoretical divergencies concerning the principles of vaginal cytology near term.

There seems to exist more important differences between our own findings and those made by Kamnitzer who insists particularly on the appearance of an eosinophilic peak shortly before delivery. We also have observed this brief appearance of eosinophilic cells at the start of the labor, but usually only in primiparas, while in multiparas it can be absent. But, as in general this eosinophilic peak appears only in cervical smears or in smears collected after a vaginal examination, we have not mentioned them in our paper, because it deals only with vaginal smears collected by our technique. And in these smears, it is very exceptional to find this eosinophilic peak. Furthermore, we have given no practical importance to this peak for the following reasons: (1) This rise of the Eosinophilic Index is only of very short duration, so that at least daily smears of the cervix are necessary in order to observe this change; (2) it is frequently absent in multiparas; (3) it is accompanied or preceeded by clinical changes such as cervical effacement or dilatation, which a trained obstetrician can detect in a more rapid manner by simple clinical examination; (4) if this peak is observed, labor starts so quickly that usually the patient has delivered before the cytological laboratory has presented its report. In the first part of this symposium Kamnitzer asks for the possible endocrinological explanation of this cytological phenomenon. We still believe that this eosinophilic peak observed in the cervical smear has an exclusively mechanical origin. Indeed, it is the cytological manifestation of the mechanical changes of the cervix at the beginning of labor. It is known that the cervical squamous epithelium does not completely follow the typical pregnancy changes of the vaginal epithelium and that very frequently part of the cervical epithelium near the external os remains covered with a keratinized superficial layer (the cervical hymen of the French authors). This layer splits off at the beginning of labor, so that it would appear in cervical smears as an increase of eosinophilic superficial cells. For this reason it is only with difficulty that we can follow Kamnitzer in his conclusion that this eosinophilic peak could be the beginning of the genital crisis of the puerperium. This means a conclusion of hormonal influence gained from a finding which in our opinion results only from mechanical changes and not from particular hormonal modifications. Kamnitzer himself seems to accept such a restriction for hormonal conclusions, since he says that the smear goes crazy as evidenced by the structural modifications of the vaginal and cervical tissues during labor. For our own conclusions concerning vaginal smears, we are rather strongly convinced that they are independent of mechanical or anatomical changes related to the beginning of labor, so that we are no longer interested in vaginal cytology for practical purposes once labor has begun.

We disagree with Kamnitzer when he says that colpocytology, alone or aided by the estrogen test, is small help for the problem under discussion. We think the vaginal smear as presented by Lemberg-Siegfried, ourselves and others, permits accurate conclusions for a much longer time. We agree that prolonged pregnancy is in most incidences a creation in the mind, not of nervous obstetricians, but of nervous patients, especially if the obstetrician has to treat patients with some intellectual niveau or those who have read too many pseudo-medical books.

For the possible causes of spontaneous induction of labor, either premature or at term, as asked for by Kamnitzer, we would refer him to the standard textbook of obstetrics for details, because this answer, if it would be complete, does not remain within the limits of this discussion.

We think that the differences with Kamnitzer discussed here result mainly from the use of a different collection technique for the smears, so that his conclusions are based mainly on the findings in cervical smears or mixed smears after vaginal examination, while we present exclusively the findings of vaginal smears before any vaginal examination.

If we now consider the main problem as presented in the main papers, we think that the rather long discussions should not let us forget the most important conclusions of this symposium, which we would like to repeat not as cytologists but as obstetricians: The practical and cytological conclusions gained in several rather important clinics of Switzerland, Czechoslovakia, France and Luxembourg and now based upon an experience of several years and several thousands of deliveries are identical: With an accuracy obtained by no other technique, the vaginal smear permits one to determine whether or not a pregnancy is at its biological term and how long it can continue without risk for the baby. This conclusion has been confirmed recently by Dr. Zidovsky of the Czechoslovakian Institute for the Protection of the Mother and Child, who has shown, in a series of 182 cases of prolonged pregnancy, that the vaginal smear gave a correct diagnosis of postmaturity in 84% of the cases while X-ray studies of the fetal bones permitted such a diagnosis only in 38%. The results of this author in the prediciton of labor in 417 deliveries are identical with our own (accuracy of 90%). The practical results of the general use of the vaginal smear in obstetrical practice are, therefore, a reduction of inductions of labor to a minimum and a sensible reduction of fetal mortality.

We would like to repeat with Nyklíček that if the vaginal smear could be applied systematically to every pregnant patient during the last month of pregnancy, it would be possible that no baby would die from postmaturity or from needless induction of labor. Experience has proved that it is possible, and we think that this result would be the best satisfaction that we could expect from our work.

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LUIS MONTALVO-RUIZ, Madrid, Spain:

To Terzano and Mezzadra: We have been only partially able to verify that which most of the authors say.

To Horálek and Sonek: The discussants are surprised over the high percentage of errors in our work; so are we. However, we think that it is necessary to describe what we have seen.

As we have already said, there are very few cases from which to draw definite conclusions. We intend to keep studying this problem and will later communicate our results.

VAGINAL CYTOLOGY AFTER RUPTURE OF FETAL MEMBRANES

B. CORNELIS HOPMAN
Miami, Florida, U.S.A.

INTRODUCTION

After comparing vaginal smears before and immediately after the rupture of fetal membranes, Goldfine (3), in a recent publication, reported observing differences in the staining quality of superficial cells. Prior to the rupture of fetal membranes, eosinophilic superficial cells were seen in clusters, together with some cyanophilic intermediate cells and a large number of leukocytes. After rupture, cells and leukocytes were less in number and showed changed staining reactions. This was explained by the washing away of superficial cells and the cleansing of the vagina by the amniotic fluid. These observations are interesting and easily verified, but their diagnostic significance may be questioned.

Other methods applied in the detection of ruptured fetal membranes have a high degree of accuracy ascribed to them. In an extensive study of the nitrazine test for the acidity or alkalinity of vaginal fluid, Abe (1) found it accurate in 98.9 per cent of the patients whose membranes were known to be ruptured and in 96.2 per cent of the patients with unruptured membranes. In a demonstration of fetal fat substance in vaginal fluid, Numers (5) reported an accuracy of 97.2 per cent when membranes were intact and 99.3 per cent when membranes were ruptured. Bourgeois' (2) over-all accuracy in the demonstration of fetal squamous cells was 97.1 per cent. Fetal hairs, according to Philipp (6), can also be demonstrated with a high degree of accuracy. Identifying characteristics permit easy differentiation from foreign material. Despite claims of a high degree of accuracy these methods, unfortunately, have not proved to be always as practical as first thought.

A method for the detection of ruptured fetal membranes should be of greatest value when the clinical diagnosis is uncertain. It is in such instances that the above methods often have not been helpful. Indicators (litmus, nitrazine, bromthymol blue, etc.) do not always encompass a pH span which includes the pH of the vaginal fluid in question. In fact, enough fluid for a test may not be available at the vaginal introitus or vulva. In addition to the fact that a false-positive alkaline reaction may be obtained if alkaline reacting materials (blood, solutions, urine, etc.) have contaminated the fluid tested, the vaginal fluid itself may be alkaline. Fetal fat substance is not readily differentiated from fat droplets in expelled vaginal epithelia. This is due essentially to the manner in which the cells are stained with Sudan III. There may be little or no difference in the tinting, and little if any reaction to the fat dye, when premature cases are involved. This method is not sufficiently specific and is far from being reliable prior to the thirty-second week of gestation.

Although fetal hairs may be recognized even among similar-looking foreign material, they occur in such small numbers that several preparations are often required to find one hair. This is time-consuming, and when time is of the essence, the effort does not seem worthwhile. Experience with the identification of desquamated and disintegrating, fetal squamous cells has shown that even trained cytologists have found it difficult to differentiate these cells from disintegrating, vaginal epithelia. Moreover, as in the identification of fetal fat substance, the identification of fetal epithelium has not been of value before the thirty-second week of gestation.

The presence of vernix caseosa cells (degenerating fetal squamous epithelium) in vaginal smears has proved to be an excellent sign for the diagnosis of ruptured fetal membranes from the twenty-fifth week of gestation. Although there have been almost five years of experience in our section of cytology in the detection of these cells, it was not until the past year that a more complete understanding of their cytologic features occurred. Now they are rarely missed if a suitable vaginal smear is obtained (4, 7).

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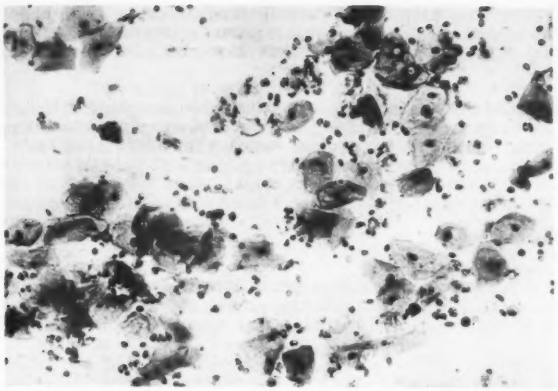

Fig. 1. Vaginal smear; fetal membranes unruptured.

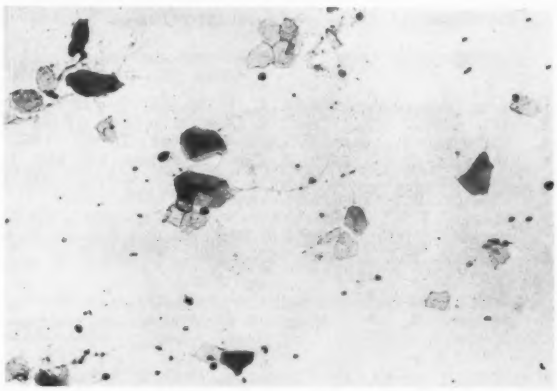

Fig. 2. Vaginal smear after rupture of fetal membranes (same patient as in Fig. 1). Cells are dispersed and red and white cells are fewer. To the right of the center is a vaginal cell visible above 2 vernix caseosa cells. At the top center are several vernix caseosa cells laying in a group. Other vernix caseosa cells are seen scattered throughout the microscopic field.

CYTOLOGY

Vernix caseosa cells are translucent, polygonal, and anucleated (Figs. 1, 2). The cytoplasm is smooth as well as translucent, being almost shadowy in appearance. With careful staining by the Papanicolaou method it may stain gray-white or light yellow, sometimes reddish, and one can see small granulations and an intricate network of communicating canals. Although nuclear remnants are sometimes still visible as uncolored material, they are essentially anucleated structures. They are polygonal in form, like that of all surface epithelia.

Because vernix caseosa cells may be confused with anucleated squamous cells, that is, vaginal epithelia that have lost their nuclei, identifying features require emphasis (Table I). Even when they are single, others are in close proximity. Important in the detection of anucleated vaginal epithelia is the presence, on the same slide, of cells depicting transitional stages of anucleation or those displaying a nuclear ghost surrounded by a halo.

Table I. Differentiation of Vernix Caseosa Cells From Anucleated Vaginal Squamous Cells

Characteristic	Vernix Caseosa Cells	Anucleated Vaginal Squamous Cells
Stain (Papanicolaou)	Gray-white, light yellow or sometimes reddish	Darker yellow
Appearance	Translucent	Opaque
Noncellular material	Uncovered	Covered by mucus, bacteria and cell rests.
Relation to other cells	Joined or layered; if single, others close by.	Single, rarely in groups
Canal system	Delicate, visible	Coarse, difficult to follow
Cytoplasm	Smooth, small granules	Coarse, heavily granular
Stages of anucleation	Absent	Present
Nuclear ghost	Absent	Present with halo

In one hundred consecutive cases there were two false negatives indicating rupture of the amnion with no visualization of vernix caseosa cells on the slides. False negatives in general may occur by slides containing too few cells, by smears not being taken at the cervical os or by infection where the leukocytes almost completely cover the cells.

There were also a few false positive cases where the membranes appeared to be intact at the time of delivery. Confusing vernix caseosa cells with squamous cells is possible, but more probable is a high rupture of the membrane with leakage of amnionic fluid into the vagina, causing vernix caseosa cells to appear on the slides with apparent intact membranes.

SUMMARY

When smears have been obtained close to the cervical os and properly stained, vernix caseosa cells may be found early in the sixth month of gestation. Since a suitable vaginal smear can be secured and stained by the Papanicolaou method within thirty minutes, and a report can be submitted within an hour, the method is not time consuming.

When the clinical diagnosis is uncertain and results with indicator substances are noncontributory (something that happens quite often), the identification of vernix caseosa cells is an excellent method for the diagnosis of ruptured fetal membranes.

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DISCUSSION

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

After reviewing the present tests for detecting rupture of fetal membranes, the author recommends the detection of vernix caseosa cells as an easy and reliable method. The most important criteria of those listed in Table I are, according to our opinion, the complete absence of any stages of anucleation and the absence of nuclear ghosts.

FRANTIŠEK HORÁLEK and **MOJMÍR SONEK**, Brno, Czechoslovakia:

Like Hopman, we found the identification of vernix caseosa cells to be a valuable aid in the diagnosis of ruptured fetal membranes. This method we are combining with an examination of the crystallization of amniotic fluid. This method is considerably shorter and simpler and its results are relatively reliable. This method which has been described by different authors (1, 2, 3) consists of putting one drop of vaginal secretion on a dry slide. After it has dried (in a few minutes) we see a very fine crystallization of amniotic fluid, which markedly differs from the coarse crystallization of cervical mucus. In order to make this fine crystallization more visible, we introduced into this method an impregnation of the specimen with 5% $AgNO_3$ for two minutes.

A possible mixture with urine, whose crystals might cause difficulties in the diagnosis, was eliminated by taking the smears with the help of a glass protector. This simple device, described in previous articles (4) makes possible the taking of smears from one place by means of a cotton swab freely moving in a glass protector.

Since it eliminates a mixture of epithelial elements of the anterior parts of the vagina and vulva, we use it also in a proper cytologic diagnosis of vernix caseosa cells.

The results of cytological examination after rupture of membranes can be divided into three groups:

1. Prompt rupture of fetal membranes in mature pregnancies.
2. Rupture of membranes in premature deliveries and miscarriages, stimulated and provoked by hormonal influences.
3. Premature rupture of fetal membranes without subsequent uterine activity.

In cases of the first and second groups the cytological pattern is similar to the pattern in the cases without rupture except for a certain spread and isolation of cells. The picture is furthermore enriched by the occurrence of vernix caseosa cells.

The cases of group three represent a premature rupture of the membranes due to mechanical causes (coitus, concussions, during a car ride, a sudden increase of intra-abdominal pressure, etc.) and further to an increased fragility of the membranes (toxemia, infection). In these cases the cytological picture preserves uniform features of pregnancy. Only the clusters are less frequent and the cells are more isolated. This picture can remain for a number of days. Only nervous impulses originating probably in the region of the contracted uterus start the hormonal mechanism of delivery. However, even the accompanying predominance of estrogens is insufficient; the deliveries are protracted and often terminate with surgical intervention.

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MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil:

We have always secured reliable cytological information when the smears were taken shortly after artificial or spontaneous rupture of the fetal membranes during labor.

Phase-contrast microscopy is definitely helpful in identifying amniotic cells and fetal squames which sometimes stain poorly with the Papanicolaou method.

In doubtful cases, however, where the presumed premature rupture of the membranes was supposed to have occurred several days before the preparation of the smears, the cytological examination was often inconclusive.

Hopman suggests an interesting criterion for the cytological diagnosis of the rupture of the fetal membranes, but he did not mention if "vernix caseosa cells" are also found in long standing cases.

We believe that the exact diagnosis of ruptured membranes in such cases is often very difficult, but cytological examination of the vaginal smears may reveal that labor is imminent.

VIOLETTE M. NUOVO, Paris, France:

We were extremely interested in Hopman's paper. It seems evident to us that it is often difficult to differentiate between vernix caseosa cells and anucleated squamous cells. We would, therefore, like to ascertain the incidence of false positives. Certainly they must be more numerous than false negatives.

We are also curious as to how often in these hundred cases Hopman found trophoblastic cells in the vaginal smear.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

Before one starts to study the vaginal smear in order to detect the rupture of the fetal membranes, the best method is first to study at the cytology of the amniotic fluid stained in the same manner as the usual vaginal smears. Only by this preliminary study can one obtain the sufficient visual memory for the detection of fetal or amniotic cells in the vaginal smear. Excellent studies of the amniotic cytology have been presented by several authors (8) whose papers should be read in the original text. In difficult cases I still recommend, if possible, the simultaneous study of amniotic fluid and vaginal content on the same slide, as proposed by Hopman in 1948 (9).

I agree in general with Hopman concerning the cytological characteristics of the fetal squames, but the absence of transitional stages towards anucleation or of nuclear "ghosts" should not be considered alone as a criterion for fetal squames, since cells of the vulvar skin can present the same cytological feature. In several slides submitted to me for final evaluation, it was mostly the presence of anucleated skin cells of the vulva which were the cause of false positive diagnosis. The contamination of the vaginal content with vulvar cells exists frequently, because the collection of the vaginal smear is frequently preceded by a vaginal examination of the patient, which should be strictly avoided prior to cytological examination. For an experienced cytologist, the differential diagnosis between fetal squames and skin cells of the mother is relatively easy, especially if the hemotoxylin-Shorr technique is used. In my experience, the most important characteristic of the fetal squames is the pale, light yellow staining of the cytoplasm which therefore presents a washed-out picture, while the anucleated vulvar cells in general stain bright red like the vaginal squames from uterine prolapse.

Hopman gives reference to a paper of Goldfine (Am. J. Obst. & Gyn. 70:109, 1955). I was rather astonished to read in this paper that prior to the rupture of the membranes, "acidophilic (red) staining superficial vaginal and cervical squamous cells were diffuse and predominating." Personally, in

many hundreds of cases studied I never could observe such a difference or the existence of numerous eosinophilic cells prior to term, so that they could be called "predominant." These findings have been presented by Lichtfus and myself in this symposium, where the reader can find a detailed description. The cause of the differences could be due to the usage of different staining techniques. As I have myself found in many cases, the vaginal smear of a normal pregnancy can show a particularly high Eosinophilic Index, if stained according to the Papanicolaou technique, while such differences never existed with the hemotoxylin-Shorr technique by which even with a less careful technique accidental variations of Eosinophilic Index are practically non-existent.

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CLOSING REMARKS

B. CORNELIS HOPMAN:

To Emmerich von Haam:

I agree that in difficult diagnostic cases the absence of stages of anucleation and absence of nuclear ghosts are the most important for differentiation between vernix caseosa cells and squamous cells. In more clear cut cases I believe the other diagnostic criteria stay more in the foreground.

To Mario de Benning Kamnitzer:

I have no experience with phase microscopy in the diagnosis of vernix caseosa cells. Vernix caseosa cells are also found in cases where the membranes have been ruptured for several days. But in these cases they are less frequent and one has to examine the slides for a longer time.

To J. Paul Pundel:

I agree that the best way to study and get a visual memory of vernix caseosa cells is to place vernix and vaginal cells separate on one slide and compare both as I described in 1948. I agree that the vulvar skin epithelia often are anucleated. However, the greater substance of these cells compared to the delicate appearance of the vernix caseosa cells mostly prevents confusion. I also stress to the gynecologists the importance of making a cytologic smear before any bimanual examination.

To Violette M. Nuovo:

Using the criteria mentioned in the paper, I don't believe that after study and training it is difficult to differentiate between vernix caseosa cells and anucleated squamous cells. False positive and false negatives happen as in every medical examination, but their occurrence is rare and does not amount to more than a small per cent. False negatives are more numerous than false positives. If the rupture of the bag has occurred some days before, it is possible that the stream of amniotic fluid has become so minimal that few vernix caseosa cells appear in the vagina. Our efforts to find them are of course restricted by the time element, resulting in some false negative reports. Reviewing these slides, after announcement of our failure has been made, reveals a few vernix caseosa cells often covered by vaginal epithelia or leukocytes and missed in our original examination. Occasionally false positives are falsely assumed as a result of a high leakage of the amnion when actually the bag of waters is still palpable. On our slides trophoblastic cells are seldom seen.

To František Horálek and Mojmír Sonek:

The method of examination of crystallization of amniotic fluid is of great interest and deserves further evaluation. If reliable, it should be an asset in our diagnostic means.

VAGINAL CYTOLOGY POST PARTUM AND DURING THE LACTATION PERIOD

MARIO de BENNING KAMNITZER
Rio de Janeiro, Brazil

Certain regressive changes in the vaginal smears commonly occur in the last month of pregnancy. They are particularly well marked in the week preceding the onset of labor (1, 2, 3, 4, 5, 6). Shortly before the clinical onset of labor, a period varying from a few to 48 hours, there often appears a veritable peak of the Karyopyknotic Index. There is a sudden shedding of eosinophilic, karyopyknotic superficial cells which seldom appear in the smears in a percentage over 30. Furthermore, the colpocytologic picture shows clear regressive changes in addition to the varying amounts of blood cells.

Since 1953 we have repeatedly described this observation (1, 6, 7), but it has not yet, to our knowledge, been confirmed elsewhere, with the exception of the above regressive changes during the last week of pregnancy.

This peak of the Karyopyknotic Index is of very short duration. As soon as clinical labor begins and progresses, the eosinophilic, karyopyknotic superficial cells tend to disappear from the vaginal smears. We feel that we have reasonable, circumstantial evidence for believing that these superficial cells are shed nearly exclusively from the squamous epithelium of the ectocervix at some moment in the "ripening" of the cervix (6, 8).

Estrogens, regardless of the amount administered, are unable to prevent these changes.

Colpocytology allows one to distinguish two clear-cut phases in the postpartum period:

(a) The genital crisis, which begins shortly before the clinical onset of labor (or abortion).
(b) The genital recovery, the beginning of which is best defined, for practical and didactical purposes, by the return of the normal (non-gravid) response of the vaginal epithelium to estrogens (1, 8).

The duration of the genital crisis depends mainly on the duration of pregnancy; after full term labor, it lasts from two to three weeks. The genital recovery is also dependent on the duration of pregnancy, but it is subject to several other factors, the main one being lactation (8).

The genital recovery which takes place during the postpartum amenorrhea is a biological period where colpocytology shows astonishing individual variations.

The average time required for the return of a normal estrogenic colpocytologic pattern is not yet sufficiently well established. According to Schellenberg and Roth (9), it takes about 45 days in non-lactating women and from 65 to 76 days in patients with fully developed lactation.

Vokaer (10) describes different features in the curves of the genital crisis and recovery in lactating and non-lactating women. He concludes, however, that on the average, regardless of the lactation, both series show normal estrogenic smears about the 45th day postpartum. De Rezende and Kamnitzer (8) found that the normal estrogenic pattern returns in non-lactating women between the 50th and 60th day postpartum and in lactating patients between the 75th and the 90th day. In isolated cases this was observed about the 35th day postpartum. In other cases the vaginal smears in lactating women remained subatrophic or even atrophic for several months. Another interesting issue in the postpartum period which requires a closer study is the so-called utero-vaginal dissociation (1, 8, 10).

Two kinds of utero-vaginal dissociation have been described (11):

(a) Atrophic vagina together with proliferative or even hyperplastic endometrium (1, 8), an association more commonly found in lactating women between the fourth and seventh week post partum.
(b) Proliferative or even hyperestrogenic vaginal smears together with a resting or atrophic endometrium (8, 12, 13), an association which seems to appear more commonly in later periods of the postpartum amenorrhea.

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WARREN R. LANG

Philadelphia, Pennsylvania, U.S.A.

The postpartum and lactation periods represent transitional stages between pregnancy and the reestablishment of regular menstrual cycles. The postpartum period is characterized by abrupt traumatic desquamation, rapidly decreasing hormone levels and secondary vaginal inflammation and infection, so-called postpartum vaginitis (1). The period of lactation, on the other hand, can be described as one of tissue regeneration and slow stabilization of hormonal processes. The vaginal cytologic smear necessarily mirrors these changes to a great degree.

The first ten days after delivery have been designed as the immediate postpartum period (2). During this time the vaginal epithelium decreases greatly in thickness. Clinically the vaginal fluid is known as lochia; this is grossly bloody for at least a week or more. Cytologically, red cells predominate and as they diminish, white blood cells gradually increase in number. Endometrial and cervical columnar cells may be seen, often degenerated and often in groups; the former may be quite large. Placental elements are occasionally found, but these, with mucus strands, in our experience are more easily detected by endometrial smears. Histiocytes are found exhibiting marked phagocytic properties; like leukocytes

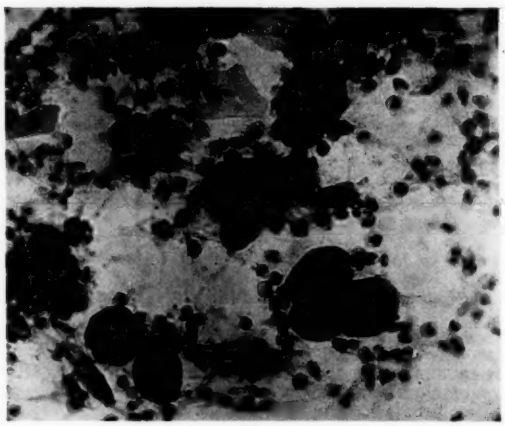

Fig. 1. Postpartum smear (400X). Four days after delivery. Note postpartum basal cells with small nuclei, perinuclear vacuolization and heavy borders. A superficial cell is present. Note also clumps of leukocytes.

they are often grouped in clusters, perhaps around degenerated cells. Although the "navicular cells" of pregnancy are not uncommon for the first few days post partum, they are soon replaced by distinctive parabasal cells, "postpartum cells." These are round or oval but may even approach intermediate cells in size. The nucleus is eccentric and generally large but pyknosis can occur; the cytoplasm may be eosinophilic. Perinuclear halo formation and a heavy peripheral cell border are frequently noted (Fig. 1).

Unless lactation fails spontaneously or is inhibited by hormonal preparations which in themselves can alter the appearance of the vaginal cytologic smear, the immediate postpartum period merges into the period of lactation (Fig. 2). Histiocytes, erythrocytes and cells from the uterine cavity are present in decreasing numbers. Leukocytes and debris presist to a varying degree. "Postpartum cells" slowly assume the morphology of normal parabasal cells and intermediate cells become prominent. It is surprising that these intermediate cells contain glycogen (2). Superficial cells are scant, presumably from the blocking effect of lactogenic hormone on the gonadotrophic hormones, thus preventing full maturation of the vaginal epithelium. After a variable period, anovular or ovular menstruation takes place with the expected changes in the cytologic smear.

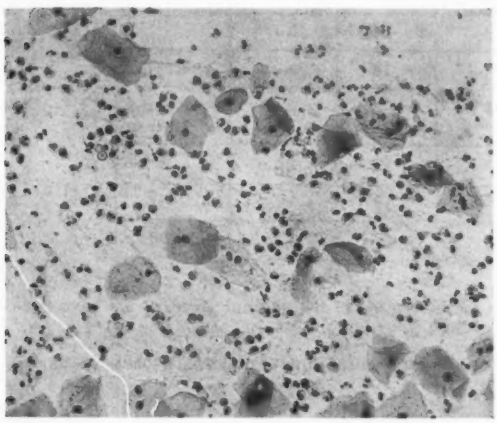

Fig. 2. Lactation smear (200X). Two months post partum; lactation present. Intermediate and superficial cells predominate. There are many leukocytes present.

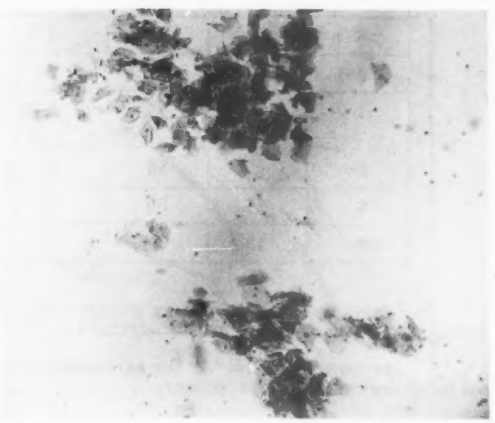

Fig. 3. Lactation smear (100X). Three months post partum. Patient did not nurse. This looks like a postovulatory smear. Three weeks later menstruation occurred.

In non-lactating women, histiocytes, leukocytes, cellular debris and endometrial elements may persist for six or eight weeks. The postpartum vaginal cells soon make way for intermediate and later eosinophilic, karyopyknotic superficial cells. The vaginal cells demonstrate more sensitivity to the rising hormone levels than the ectocervical cells do (3). Several months after delivery menstruation usually occurs. As with lactation, this may or may not be preceded by ovulation (Fig. 3). At about this time also Döderlein bacilli may reappear and be noted on the smear.

Although the above brief description presents the overall pattern of cytologic findings, there are often variations in the smear, especially from local infections – primarily Trichomonas vaginalis. Candidal vulvovaginitis usually subsides post partum.

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OTAKAR NYKLÍČEK

Náchod, Czechoslovakia

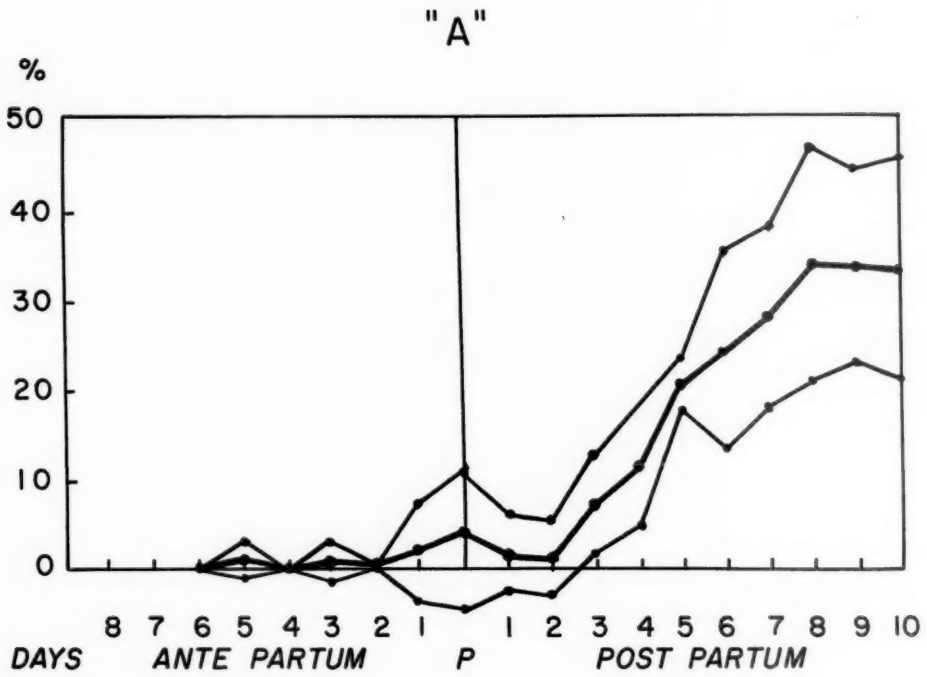
It is our opinion that it will be instructive to present an uninterrupted series of vaginal smears before and during delivery, in order to show the dynamics of cytological reactions to the drastic hormonal changes following birth.

For this purpose we have made a detailed study of 210 vaginal smears from 30 women taken during a 19-day period, that is, eight days before and ten days after delivery. We procurred the vaginal smears either daily or every other day. The results were entered in vaginal cytograms, one of which is presented here.

Date taken	A	B	B_N	C_1	C_2	L	Other Elements
5	6	33	18	27	16	--	
3	8	16	20	38	18	--	
1	19	28	6	22	25	--	
Partus	22	26	4	19	29	--	Erythr. Uter. el.
1	17	19	0	31	33	--	"
2	11	30	0	29	30	--	"
3	21	32	0	28	19	--	
4	18	37	0	32	14	--	Erythr.
7	28	38	0	26	8	--	Erythr. Uter. el.
8	23	46	0	19	12	--	"
9	16	30	0	36	18	--	"
10	13	38	0	25	24	--	Erythr.

In order to give a clearer picture concerning the dynamics of the vaginal picture, we have presented the values of the vaginal cytograms from all 30 women in graphic form.

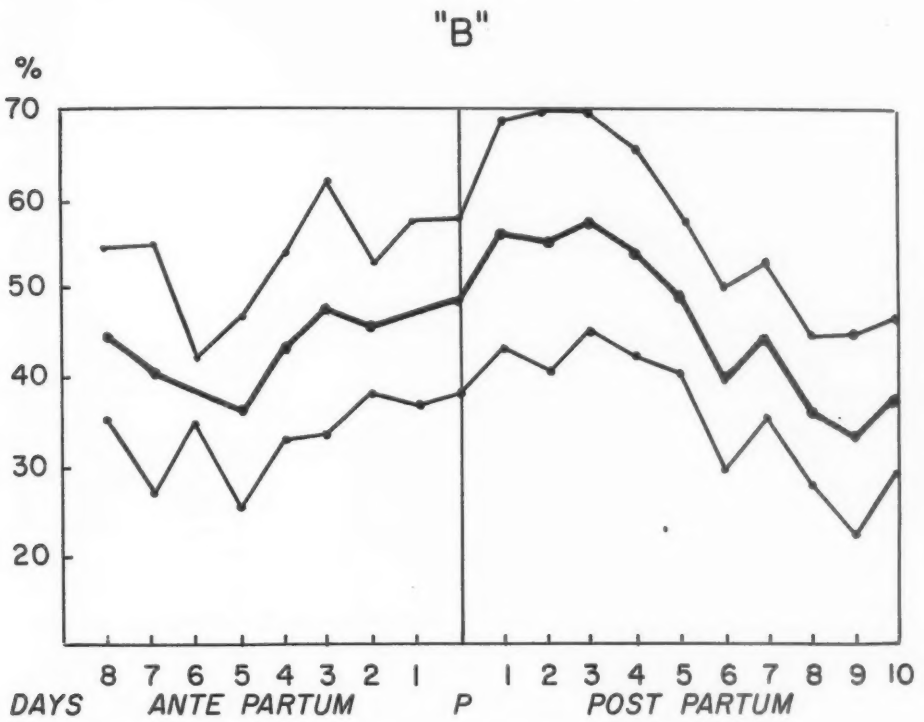
In the first graph "A" the parabasal cells of the vaginal epithelium are shown. These cells are not found during normal pregnancy.



Graph of the arithmetical mean of "A" with standard deviation.

Arithmetical mean of the sum of all values in this graph was 0% during the seven days before delivery, 1.58% on the day immediately preceding delivery. It was 1.33% on the first day following delivery and rose to 28.22% on the seventh day. On the tenth day of puerperium it was 34.00%. We have recorded then a strikingly rapid increase of the cells of this group in the first ten days following delivery.

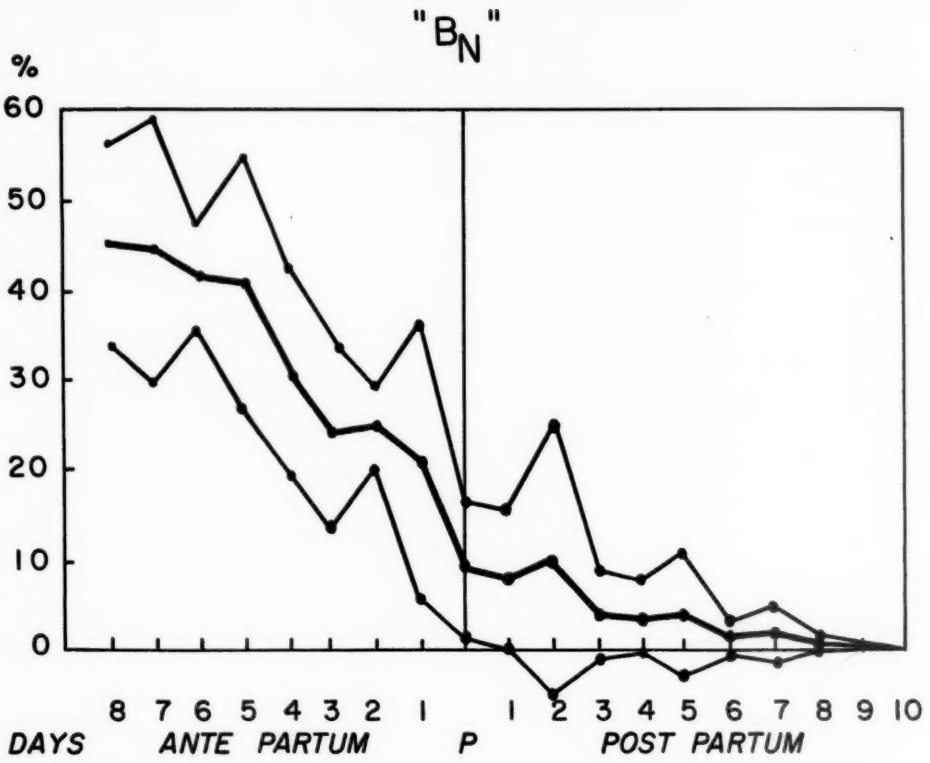
A graphic transcript of "B" concerning the cells of the normal middle layer is presented here.



Graph of the arithmetical mean of "B" with standard deviation.

The mean of these cells is 40.61% a week preceding delivery; on the first day after delivery the mean is 44.44%, and on the tenth day of puerperium the mean is 38.20%. The cells of this layer occur roughly in the same numbers during the week preceding delivery and during the ten days following it and represent the largest group of cells.

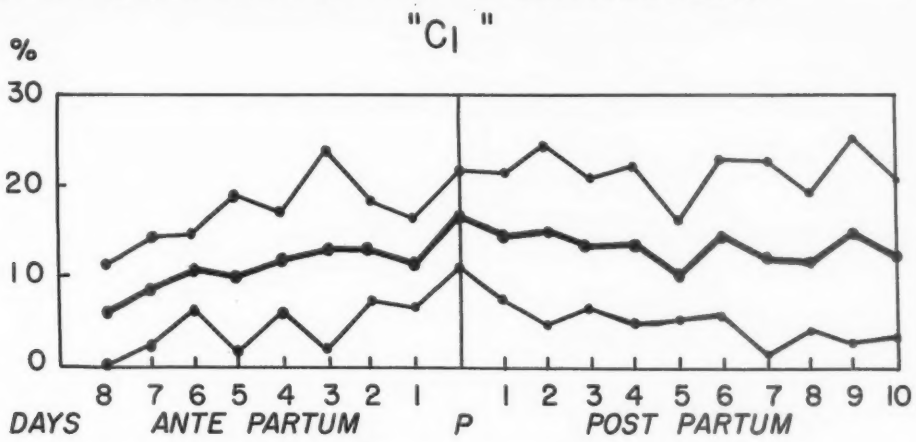
A graphic transcript of "B_N" is presented here. We include the navicular cells of the middle layer which account for 75% of all cells in the smears in advanced pregnancy. Their number diminishes during the last two weeks preceding delivery, and they quickly disappear during the several following days.



Graph of the arithmetical mean of "B_N" with standard deviation.

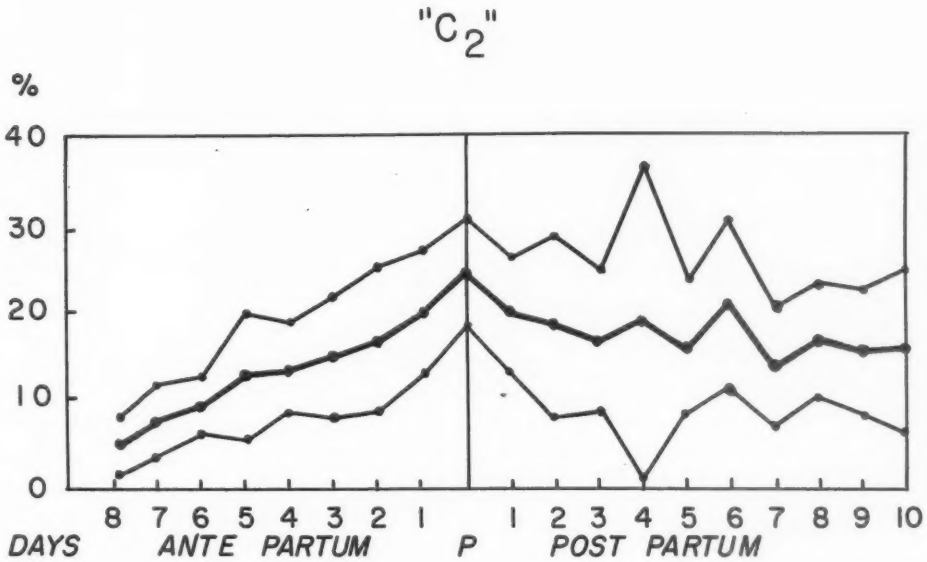
They account for 44.30% in the first seven days before delivery. It diminishes to 22.58% on the day immediately preceding delivery and to 7.67% on the first day following it. A week following delivery they are represented by 1.11%, and on the tenth day after delivery none of these cells can be found in any of our women.

A graphic transcript of "C_1" shown here represents the cells of the superficial layer with a vesicular nucleus. They are found on the average in 8.31% of the cells a week before delivery. Their number is slightly increased to 11.42% on the last day preceding delivery. On the first day following delivery it is 14.33%, a week later 12.44%, and on the tenth day after birth it is 12.00%.



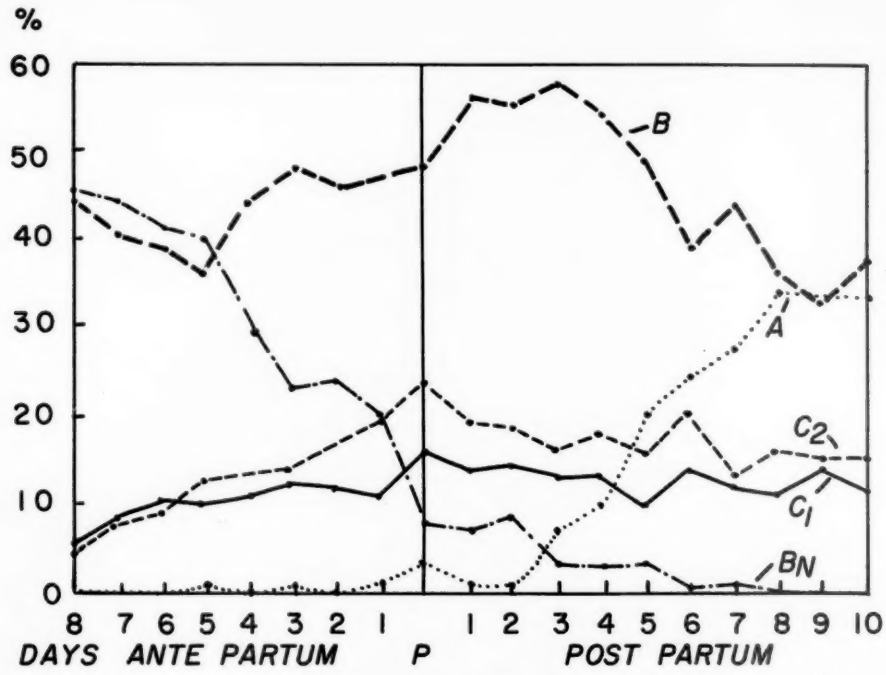
Graph of the arithmetical mean of "C_1" with standard deviation.

A graphic transcript of "C_2" is presented here. It concerns the karyopyknotic cells of the superficial layer. These cells, as we know, are rarely found in smears of advanced pregnancy. We have found them on the average of 7.54% on the seventh day before delivery, 19.42% on the day immediately preceding the delivery, 19.73% on the day immediately following it, 13.78% a week after birth, and 15.80% on the tenth day following delivery.



Graph of the arithmetical mean of "C_2" with standard deviation.

In the following summarized graph are presented the arithmetical means of all five graphs of vaginal cytograms from the 30 women.



Graph of the summary of the previous 5 graphs.

We believe that this is the best way to make clearly evident the dynamics of the vaginal smears during the period in question. In order to explain the hormonal circumstances in puerperium, which so essentially influence the target organs, i. e., the vagina and the endometrium, it is necessary for us to look back at pregnancy and to go through the hormonal combination once more. The enormous quantity of estrogens produced in pregnancy influences the vaginal epithelium by a strong proliferation of the middle layer. If, for this reason, we find no hyperestrogenic manifestations, it is explained by the fact that the progesterone, produced in great concentrations, is converting the active estradiol and estrone into the less active estriol. This is manifested by smears of the so-called "type of advanced pregnancy smear." Immediately before delivery, through a decline of the level of progesterone, this conversion disappears and the level of activity rises. It manifests itself in the cytological picture of the so-called "type of smear preceding delivery." The separation of the placenta after the birth of the fetus is followed in a very short space of time by sudden endocrinological changes. The aim of the endocrine glands of this period is to promote lactation. From the various unsubstantiated hypotheses the most probable seem to be those which maintain that active estradiol helps the hypophysis to produce prolactin. The ovarian hormones then are produced in very low concentrations as in the other amenorrheas. This is clearly visible in the above mentioned regression of the sensitive tissues, such as the vaginal epithelium.

HANNAH PETERS

Copenhagen, Denmark

Vaginal smears taken during the postpartum months on lactating women fall into three smear type groups:

(1) The atrophic smear (estrogen deficient state).
(2) The intermediate smear (the epithelium is stimulated to grow up to the intermediate layer).
(3) The mature smear (adequate estrogen stimulation).

One might expect to find the atrophic smear during most of the amenorrheic, postpartum months and the mature smear perhaps only after the menstruation has been reestablished. However, vaginal smears, taken during the lactation period on women whose menses have not yet returned, actually show a different distribution: the atrophic smear is only seen in the first three postpartum months; in later months, only intermediate and mature smears are found even though the lactation amenorrhea does persist. Smears taken at the same time from the ectocervix show a different distribution of types. Atrophic cervical smears can occasionally be seen up to the 12th postpartum month in amenorrheic, lactating women. When vaginal and cervical smears, taken at the same time on the same patient, are compared, it is noted that the cervical smear usually lags behind the reaction of the vaginal smear; a woman whose vaginal smear is mature might still show an intermediate or even atrophic cervical smear. This suggests a differential sensitivity of the vaginal and cervical epithelia to hormone stimulation. The sensitivity to hormones of the cervical epithelium seems to lie between the sluggish response of the endometrium and the rapid response of the vaginal epithelium.

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ROGER VOKAER
Bruxelles, Belgium

Vaginal postpartum cytology is considered in three different phases:

1. IMMEDIATE POSTPARTUM PERIOD

The vaginal smear rapidly loses the characteristics of pregnancy cytology. Navicular cells of pregnancy disappear little by little, whereas leukocytes, erythrocytes and numerous histiocytes invade the smear. Around the fifth day a few typical cells appear: they are oval or round elements of small size with globular nuclei, with the cytoplasm containing a few vacuoles. A few atrophic eosinophilic cells are also seen similar to those observed in senility. These cells and the navicular cells of pregnancy create the typical "immediate postpartum" cytology. At this period authors agree that it is impossible to tell whether or not the woman nurses. We have never noticed any cells from the endocervix or the intra-uterine cavity. It may happen that such elements are present in smears, but very often they are not. Likewise, we have seen nothing that looks like remnants of exfoliated decidua; thus, we think that actual regression probably dominates the elimination phenomenona of the necrotic decidua.

2. EARLY POSTPARTUM PERIOD

Lactation: Around the tenth day fairly large cells are observed, of the intermediate type with large and round or oval nuclei and cyanophilic cytoplasm. Around the 15th - 20th day the size of the cells decreases (4 or 6 times), whereas nuclear size increases (1-1/2 times). These peculiarities characterize the typical subatrophic vaginal smear during lactation (Pundel and Van Meensel). Whether or not histiocytes are present cannot be determined. After this phase (characterized by atrophic cytology) and around the 25th day one observes changes similar to "slightly estrogenic reaction," clearly expressed by the presence of superficial cells (rarely eosinophilic) of which the nuclear diameter is about 6 to 9μ. On the 40th day cytology is of the pure follicular type and entirely similar to what can be observed on the eighth day of the normal menstrual cycle. Finally, around the 45th day we have often observed an intense estrogenic reaction with exfoliation of large, singly lying, karyopyknotic, eosinophilic superficial cells.

No Lactation: When lactation has been stopped by administration of estrogens, exfoliated cells become subatrophic and even atrophic. The round and globular nuclei are slightly smaller than they are during lactation. But from the 15th day on, changes occur and lead to a "mixed postpartum cell type": in addition to the presence of numerous atrophic cells, small intermediate cells and even superficial cells of small size appear. Signs of a very progressive estrogenic stimulation appear, leading to exfoliation of intermediate and superficial cells around the 30th day. These cells present folded edges and wrinkled cytoplasm and the nuclear diameter decreases (5μ). The Eosinophilic Index increases and around the 45th day the smear pattern is the classical estrogenic one. If during the immediate postpartum period the vaginal cytology was not influenced by estrogens, the epithelium becomes sensitive again about fifteen days after delivery. The estrogenic proliferative type occurs much earlier. Because of the administered estrogens one cannot speak of a typical lactation smear (Van Meensel, 1952).

We cannot definitely appreciate the quantitative value of the various estrogens we have been using because of the small number of cases. However, it does not seem impossible that the ethinyl-estradiol has a great influence on the regeneration of the epithelium. In dosages that are actually considered as equivalent (1 mg. distilbene = 20γ of ethinyl-estradiol) it seems to us that ethinyl-estradiol was particularly active. We have observed that the receptivity of the vagina to estrogens was decreasing between the first and the 15th day of the post partum period. This is well proven by the fact that vaginal cytology of the first ten days of the postpartum period does not vary with or without the administration of estrogens. Van Meensel, on the contrary, has observed cytologic alterations already after the sixth day: superficial cells, Eosinophilic Index between five and 30, Karyopyknotic Index between 30 and 65. Although

he has not done simultaneous vaginal biopsies, he comes to the conclusion that an evident reactional "inertia" of the vagina exists during the postpartum period. In eight of our cases dosages of ten times 5 mg. of dienestrol did not succeed in increasing the Eosinophilic Index above 12. The Karyopyknotic Index as measured by our technique (nucleus: 5μ or less on the immersion objective, and ocular 6X) has never exceeded 10. Outside of the post partum period such therapy always induces, however, an increase of both indices.

After the 15th day the estrogenic reaction obtained by even very small dosages of estrogens leads to alteration of the vaginal cytology. The inertia observed after delivery lasts only two or three weeks. After that time the epithelium recovers its normal reaction potentialities.

3. LATE POSTPARTUM PERIOD

If there is no lactation, the cyclical alterations of the vaginal cytology recur, whereas if lactation is maintained the cellular maturation is inhibited, leading to the exfoliation of intermediate cells. "The appearance of the smear is then quite specific: it contains many intermediate or basal cells, cyanophilic or eosinophilic, fairly large and roundish. These cells are characterized by hypochromatic nuclei and cytoplasm which is particularly rich in glycogen." (Pundel) These characteristics persist during the entire lactation period.

If, during the early postpartum period (11th to 45th day after delivery) one compares the vaginal cytology or vaginal histology with endometrial histology, one notices a dissociation in time between the endometrial evolution and that of the vaginal epithelium. Around the 20th - 25th day (especially when there is lactation) atrophy of the vaginal epithelium is observed, whereas the endometrium has perfectly recovered and exhibits proliferative changes. After the 45th day after delivery, in certain cases where lactation is prolonged, the cellular layers of the vagina are numerous, whereas the uterine mucosa is not well developed. I believe that the appearance of the often anarchical, proliferative endometrium indicates that the tissues are trying to cicatrize (whereas the vaginal mucosa is at rest) and this is without any hormonal influences.

Around the 25th day the endometrium begins to proliferate, while stimulation phenomenona are observed in the vagina. This must be the time when the ovaries function again. It does not seem necessary to us to invoke a difference of receptivity to estrogens in order to explain the differences of proliferation of endometrium and vagina. The endometrium, considerably damaged by obstetrical trauma, proliferates first by simple cicatrization, then by hormonal stimulation. The vagina (less damaged) awaits hormonal stimuli in order to start its reconstitution.

Finally during the late post partum period (in lactation), a well-developed vagina can be observed while the endometrium is hypotrophic. To explain such different reactions Van Meensel believes that one must consider the influence of other factors which may be associated with the histophysiology of the vaginal epithelium. It seems very difficult, if not impossible, to explain most variations of receptor sensibility at this time.

DISCUSSION

FRANTIŠEK HORÁLEK and MOJMÍR SONEK, Brno, Czechoslovakia:

On the basis of 75 serial cytodiagrams we studied the cytological picture of early puerperium and its relation to lactogenesis. Although it is commonly stated that the smears of the early puerperium do not depend on lactation (up to ten days after delivery), we have come to the conclusion that there exists a certain dependence of the cytological changes on lactogenesis (onset of lactation), which should be distinguished from galactopoesis (proper secretion of milk). In accordance with Kamnitzer we found a considerable individual variability in the cytological picture of the puerperium. The cytodiagram, however, can be divided in most cases into two phases:

1) The phase of a relative predominance of estrogens in the smears, starting a certain period of time before delivery and reaching its highest point shortly before the onset of labor. This phase lasts in most cases until three to five days after delivery.

2) The phase of a sudden drop in the level of estrogens and the predominance of parabasal lactation cells and intermediate cells, which reach a considerable degree of variability in their size and shape. The change into this second period is coordinated with the time of the maximum turgor of the breast and with the proper lactogenesis.

This conclusion would on the whole correspond to the course of Nyklíček's curves, the only difference being that we have found that the parabasal cells are increasing to the detriment of the superficial cells. Therefore, it seems that not only an irritating influence of estrogens is necessary for lactogenesis, as Turner's theory suggests, but also a following drop of the estrogenic level, thus releasing the activity of prolactin.

This theory is supported by the following facts:

1) In deliveries where the first phase was cytologically found to be insufficiently developed (in protracted deliveries, terminated often by surgical intervention, in deliveries with a premature rupture of the membranes), the dividing line of lactogenesis is slight and galactopoesis is not abundant.

2) Where estrogen was administered during delivery in large quantities to induce labor or to intensify uterine activity, the phase of the relative predominance of estrogens is prolonged and lactogenesis starts later.

3) In accordance with Vokaer we found that estrogens administered in the early puerperium do not raise the Karyopyknotic Index. In many cases we found that if estrogen was administered in small doses (1-2 mg) within three days after delivery (especially in deliveries with an insufficient relative predominance of estrogens), administration of estrogens might have imitated the natural provocation of lactogenesis. Thus, a paradox reaction to estrogens often occurred; deep and intermediate cells appeared in large numbers and lactogenesis resulted. This paradox reaction reminds one, to a certain degree, of the paradox reaction to estrogens described by Pundel in the treatment of threatened abortions.

We are uncertain whether the cytological picture of the puerperium could be called atrophic or subatrophic, as it is called by most speakers. The lactation cells differ entirely from the parabasal cells in common atrophic smears (e.g., postmenopausal) both by their special morphological characters (relatively smaller nucleus, heavy peripheral cell border) and by their cytochemical qualities (high content of glycogen). We believe that we do not deal with atrophic cells, but rather with cells with a considerable proliferative potency.

HERBERT E. NIEBURGS, New York, New York, U.S.A.:

The reactions of the vaginal smear immediately preceding and following labor are well outlined and do not require further comment. The increase of karyopyknotic superficial cells which occurs between the second and third month post partum in the amenorrheic lactating woman has been brought out by the contributing authors and appears to be in contrast to the state of the endometrium, which is hypertrophic, as reported by Vokaer. This observation demonstrates a physiological aspect of the vaginal epithelium which is usually underemphasized in the hormonal interpretation of vaginal smears. The presence of an increased number of karyopyknotic cells in vaginal smears of amenorrheic women is not necessarily an indication of adequate estrogenic function. Apparently, the effect of estrogen on the vaginal epithelium is not determined by the degree of estrogen activity alone, but also by the duration of estrogenic stimulation, though this may be subnormal. Thus the linking of certain functional disorders to so-called "hyperestrinism" on the basis of the vaginal smear findings may be misleading.

The observations of Hannah Peters and Lang concerning the lower sensitivity of the ectocervical epithelium to estrogenic stimulation than the vaginal epithelium is in agreement with my own findings.

Nyklíček's explanation for the lack of karyopyknosis during pregnancy is interesting. I should like to ask Nyklíček for further information in regard to the absence of karyopyknosis during pregnancy because of conversion of estradiol and estrone into estriol by progesterone,

J. PAUL PUNDEL, Luxembourg, Luxembourg:

The reports presented by the main speakers are rather identical except for some minor differences which seem to result either from different material or from a lack of correct distinction between various periods in the lactating and non-lactating patient. I think that a correct classification, as done by Vokaer and Lang, is most important, not only for the study of the vaginal cytology during the postpartum period, but also for the correct understanding of the hormonal background of this period.

A. The immediate postpartum period is characterized by the loss of the pregnancy modifications of the vaginal epithelium, as well as of the endometrium.

B. In the early postpartum period (10th to 25 - 35th day) we have to distinguish between lactating patients and those who do not nurse their baby.

In non-lactating patients we find a progressive return to the non-pregnant pattern of the vaginal epithelium, to normal cytology and a restoration of the non-pregnant endometrium.

In lactating patients the vaginal involution continues for a distinct period of time, but only in exceptional cases will the epithelial regression reach such degrees that one could speak of true atrophy. During this time the endometrium shows a different evolution: it shows proliferation contrasting with the regression of the vaginal epithelium.

C. The late postpartum period is very short in the non-lactating woman where it is characterized by the return of the normal cyclic changes of the vaginal and uterine epithelium. However, in some cases menstruation does not occur after the first "vaginal epithelial cycle," and this can even be followed by one or more other "vaginal epithelial cycles" before the first menstrual bleeding occurs after delivery. But in the latter cases the vaginal changes are not as pronounced as in the normally menstruating patient (subfunctional cycles with incomplete endometrial changes).

In the lactating woman the vaginal epithelium shows a particular proliferation resulting in the development of a thick intermediate layer without formation of a superficial karyopyknotic layer. These particular epithelial modifications produce a particular vaginal smear pattern which I, with Van Meensel, have called the "lactation smear type." This smear type will persist as long as the ovaries do not show a return to normal function resulting in the first bleeding after delivery. On the other hand, the endome-

trium during this period shows discontinuance of proliferation or even a tendency towards more or less pronounced atrophy, contrasting now with the proliferation of the vaginal epithelium.

This "lactation smear type" often is considered as evidence of vaginal atrophy, but such atrophy does not exist. The first peculiarity which should prevent such a conclusion is the high glycogen content of the vaginal cells of the "lactation type," resulting from hyperplasia of the vaginal epithelium without complete differentiation to karyopyknotic layers. As pointed out by Van Meensel, Vokaer and myself, there remains an interesting research problem concerning the etiology of this particular vaginal proliferation and the various behaviors of the endometrium during this period. These vaginal and endometrial reactions are very similar to those observed after marked androgen treatment with the exception of the eosinophilia of the intermediate cells. Since we know now that the adrenal cortex plays an important role in the maintenance of lactation, it could be possible that these particular vaginal and endometrial reactions during the late postpartum period in the lactating woman are evidence of some particular hormonal function of the adrenal cortex.

YOSEUP S. SONG, Providence, Rhode Island, U.S.A.:

The detailed cytological pictures seen during the postpartum and lactation period seem well illustrated by the main speakers, and I have no further comments to make. However, in the series of our screening project, about 1% of the smears taken from postpartum cases were classified as atypical, and we recommended repeat cytological smears in three to six months or until the normal cytological picture returns.

GUILLERMO TERZANO and JOSÉ MARIA MEZZADRA, Buenos Aires, Argentina:

It has been rather difficult for us to evaluate vaginal smears during the early postpartum period. This is because the smears appear "dirty," with a large amount of erythrocytes, numerous leukocytes and histiocytes, together with endocervical and endometrial cells (many of them poorly preserved), necrotic cells, cellular debris, etc.

After a few days a vaginal smear pattern which differs from that observed during pregnancy can be recognized. The smear is composed mostly of non-navicular intermediate cells, cells of the outer basal layers (possibly endocervical) and the well-known postpartum cells described by Papanicolaou.

During the late postpartum period we have observed a) in lactating women: isolated intermediate and superficial cells (the latter not in higher concentration than three to five percent, using Papanicolaou's staining technique). (The presence of relatively large nuclei should be noted even in cells of the superficial type.) b) in non-lactating women: progressive changes toward the estrogenic smear type become noticeable around the sixth week. (It is almost impossible for us to state this conclusively because most of our patients in the latter group received estrogenic therapy.)

CLOSING REMARKS

MARIO de BENNING KAMNITZER:

The classification of Vokaer (10) is an excellent clinical classification which corresponds closely to the anatomical and functional events of the post partum of full term or near-term deliveries. His histologic studies on the regeneration of the vaginal epithelium (10) in the "immediate" and "early" postpartum periods are also most convincing of the correctness of the classification, and what is very important, they show an unmistakable distinction in the regeneration between the lactating and non-lactating patient.

It is true that colpocytology permits, in general, recognition of the existence of the three periods quoted by Pundel. The definition of the immediate post partum, in terms of exfoliative cytology, does not seem very fortunate to us, because the normal pregnancy smear pattern is usually lost shortly before or during labor. We also feel that in most cases it is difficult, if not impossible, to make a correct colpocytologic distinction between lactating and non-lactating cases before the third postpartum week. This might be explained by the fact that functional cytologic pictures do not always have an easily recognizable histologic counterpart.

The wide individual cytologic variations, furthermore, which occur mostly in lactating patients, in our opinion do not permit an establishment of a uniform chronology of the cytologic changes which ultimately lead to the "reconsitutio quad ante graviditatem." This difficulty must necessarily confer a provisional character to the classification of the cytologic postpartum changes.

Since we recognized that there is no definite and constant relationship between the colpocytologic changes and the time consumed for their accomplishment, we preferred to speak of a "vaginal crisis" and a "vaginal recovery" (in cytologic terms), because both processes may be recognized after the interruption of pregnancy at any stage of its development. The lactation changes, so well described by Pundel, we consider the most important intercurrent event in the process of vaginal recovery to "reconstitutio quod ante."

The regeneration of the vaginal epithelium in the postpartum period and the cytologic change to the lactation pattern apparently do not need the presence of the ovaries. This we could observe in a case of postcesarean radical hysterectomy where the patient eventually presented all the familiar regressive

and proliferative changes, exhibiting in the sixth postpartum week a beautiful normal "lactation smear type" which is supposedly associated with normal estrogen levels.

WARREN R. LANG:

Dr. Song mentions that atypical-appearing cells may be found in the postpartum period. This is both true and important to remember. It is not possible to completely categorize and compartmentalize the vaginal smear changes after delivery, because, just as in all biological processes, the sequence of changes may vary qualitatively and quantitatively among different patients. However, the general principles of Pundel seem most valid. Dr. Nieburgs makes the statement that "the linking of certain functional disorders to so-called hyperestrinism on the basis of the vaginal smear findings may be misleading." This must constantly be born in mind since the vaginal smear is an excellent but not necessarily a perfect mirror of endocrine function. The dual research problem mentioned by Pundel, namely the study of the peculiar postpartum lactation cell and the activity of the endometrium at this time, should furnish fruitful material for a serious minded investigator.

OTAKAR NYKLÍČEK:

I take the liberty of adding only a few short annotations to the text of the discussants.

To Terzano and Mezzadra: It is rather difficult to evaluate vaginal smears during the early postpartum period because the smears look dirty, with large amounts of erythrocytes and numerous leukocytes and histiocytes.

It is of course true that such a picture may be found during the early postpartum period, but it is possible to evaluate such smears, according to our experience, for the cells are well separated from each other and can be well stained and therefore, may be placed into their respective layers of the vaginal epithelium according to their morphological and tinctorial properties.

It is interesting that the secretion of estriol is so great only during pregnancy. It is certain that mainly estradiol is changed into estriol by the influence of progesterone. Shortly before delivery the placenta degenerates considerably and the secretion of progesterone is decreased. At the same time the amount of estriol in the urine is lowered, whereas the amount of estradiol and estrone rises. This takes place at the time when the fetus is already ripe, in order for the estradiol to make the uterus sensitive for pituitary oxytocin.

Progesterone becomes "a protecting" hormone during pregnancy, for the "active" components of estrogen (estradiol and estrone) are converted into "inactive" estriol, which is not "strong" enough to bring about complete differentiation of the vaginal epithelium.

Undoubtedly, women need this type of steroid hormone during pregnancy for important tasks.

The small activity of estriol, therefore, is not apt to cause karyopyknosis either during exogenic application in menopause or in castrated animals.

HANNAH PETERS:

Commenting on Nieburg's and Pundel's remarks that in lactating amenorrheic women the vaginal smear may show an intermediate or mature type whereas the endometrium may show discontinuation of proliferation or even atrophy: It has been our experience in Indian women, who have an unusually long period of lactation amenorrhea, that the endometrium may remain stationary in the early proliferative (inactive) stage for many months though the vaginal smear shows progressive proliferation to the intermediate or even the superficial layer. I would agree with Nieburgs that care must be exercised in drawing conclusions from the appearance of the vaginal smear along to the functional state of the endometrium without taking into consideration that the vaginal epithelium apparently reacts much more sensitively to hormones than the epithelium of the cervix and especially that of the endometrium.

ROGER VOKAER:

The various discussions concerning this question lead to the conclusion that all the authors agree with the main speakers concerning the principal facts and observations. They only concerned themselves about some questions of detail and interpretation.

Nieburgs, for example, insists upon the morphological contrast between endometrium and vaginal epithelium, in order to point out the fact that the "presence of a great number of karyopyknotic cells in vaginal smears of amenorrheic women is not necessarily an indication of adequate estrogenic function."

This is not exactly our point of view. We think that the vaginal epithelium does react in a very special and always identical way; as for the endometrium it may proliferate in the early postpartum period under the influence of cicatricial stimuli.

On the other hand, we are in full agreement with Song, Terzano and Pundel and appreciate their interesting remarks.

Concerning Horalek's study of vaginal cytology and its relationship to lactopoiesis, we have no opinion, since we have not studied that particular aspect of the problem.

VAGINAL CYTOLOGY IN ABORTION

GIUSEPPE DELLEPIANE
Torino, Italy

In agreement with many authors, one can state that vaginal cytology represents, from the practical standpoint, a useful test insofar as diagnosis, prognosis and treatment of the progress of a pregnancy is concerned.

I do not think that it is necessary to outline the characteristics of the vaginal smear during the first months of pregnancy, nor the hormonal factors which determine them, for all of this has already been well described.

According to our experience, however, I believe that one should discuss the pathological cytological characteristics indicating poor prognosis of pregnancy, whatever the clinical symptomatology may be. Such characteristics are: an Eosinophilic Index higher than 35, with navicular cells rare; red blood cells; abundant mucus; intermediate cells, such as the ones seen after delivery; predominance of superficial cells (isolated or in small clusters with marked signs of cytolysis) over intermediate and basal cells; predominance of cervical and endometrial cells as well as of ovular debris and histiocytes, when abortion is well under way.

If these cytological characteristics are present, we consider the abortion as irreversible, therefore not to be treated medically.

When, on the other hand, vaginal cytology shows an Eosinophilic Index and a Karyopyknotic Index lower than 35 and 45 respectively, along with red blood cells and numerous navicular cells, with rare signs of cytolysis and no cells of the "postpartum" type, then the pregnancy can be considered as having a good prognosis and therefore, to be treated medically.

In these cases, besides the usual therapy (bed rest, antispastic drugs, etc.), hormones are administered (estrogens alone or estrogens and progesterone). The daily dosages of estrogens vary from 4 to 40 mg, whereas the daily dosages of progesterone vary from 20 to 60 mg. In quite a few cases we also use microcrystals of progesterone (200 mg) and of estrogens (10 mg).

During the time of therapy cell studies are performed daily. This is also done in order to control the efficiency of hormone therapy.

In cases with poor prognosis of the pregnancy we notice, 24 hours after the therapy has started, increased Eosinophilic and Karyopyknotic Indices, further increases of the number of superficial cells, both eosinophilic and cyanophilic, and decrease of the navicular and intermediate cells.

If the prognosis is good, one notices, besides the improvement of the clinical symptomatology, a decrease or at least persistence of the initial values of the Eosinophilic and Karyopyknotic Indices, increase of the navicular or intermediate cells, and disappearance of the red blood cells, with a gradual recurrance of the typical pregnancy cytology.

All those cases are excluded where the local findings or general factors by themselves may be such as to make the symptomatology of the abortion an irreversible one.

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CLARICE do AMARAL FERREIRA
Rio de Janeiro, Brazil

In the majority of cases there is a typical vaginal cytology picture during pregnancy. The interruption of pregnancy ought to change this typical picture. The abortion can be recognized cytologically by the general aspect of the picture more than by specific cells, even when there are certain types in certain cases, as stated by Papanicolaou (1).

In cases of abortion, in general, there are two pictures in the vaginal cytology: In the first, when the hormonal (progestational and gonadotropic) deficiency apparently induces imminent abortion, the estrogenic hormone imbalance by progestational deficiency stimulates the occurrence of a specific aspect: eosinophilia and karyopyknosis (superficial cells) increase over 10%. When there is blood present, great numbers of leukocytes and histiocytes, and especially when the Karyopyknotic and Eosinophilic Indices increase (2), we can predict the imminence of the abortion if adequate treatment is not given.

The second picture is a result of the death of the ovum, whether expelled or not, and follows the first picture. As in the postpartum period, the superficial and intermediate cells are rapidly substituted for by parabasal (or deep) cells. There sometimes appears a special type of small, round cells, with a marked cellular membrane, sometimes eosinophilic, with pyknotic nuclei, called "postpartum cells" (Papanicolaou). As after the death of the ovum (or its total expulsion) the estrogenic hormone level decreases in the blood. This cellular aspect is understood. Finally one finds great numbers of leukocytes and histiocytes, cellular debris and sometimes trophoblastic cells in these smears.

In cases of artifically induced abortion the typical cellular picture appears later, because the corpus luteum is still functioning at the moment of fetal expulsion (2). In some cases of provoked abortion by uterine curettage, Papanicolaou observed the presence of muscle fibers in the vaginal material; this may serve as an index in the medico-legal diagnosis of criminal abortion.

Thus, when abortion is imminent, the normal typical vaginal cytologic picture of pregnancy changes from predominant intermediate cyanophilic cells to a high incidence of superficial karyopyknotic, eosinophilic cells. Afterwards, when abortion has taken place or after the death of the ovum, the picture changes again to the predominantly deep-layer cells, cyanophilic and sometimes with a typical cellular aspect. Sometimes the first change to the eosinophilic type is not present and we see rapid appearance of the second picture. The "clean" aspect changes also to a "dirty" one, with plenty of leukocytes, histiocytes, cellular debris, etc.

These are the "common" or "typical" pictures; but it is well to remember that one should not request that the cytologist furnish a differential diagnosis as to whether or not one deals with an abortion. Cytology reflects only the reaction of the vaginal tissue to the hormones, depending upon the hormonal situation and also on the individual vaginal response.

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OTTO STAMM, V. RAWYLER AND GUSTAVE RIOTTON
Geneva, Switzerland

Definitions:

Threatened abortion - pregnancy accompanied by one or more of the following symptoms: hemorrhage, contractions (confirmed by palpations), or dilatation of the cervical canal.

Pathological smear - elevation of the Eosinophilic Index (E.I.) and of the Karyopyknotic Index (K.I.) above the norms indicated by Pundel and Gaudefroy, in the absence of local irritation. We will not take into consideration here the other smear elements which can vary during threatened abortion and which we will discuss in another article.

In the scope of this brief exposé we will limit ourselves to the problem of hormonal evaluation of the cytological modifications in the threatening abortion.

I. Threatened abortions with pathological smears

In 110 cases of threatened abortion (62 cases, that is 56%), before the beginning of hormonal treatment, an elevation of the E.I. above normal levels was present. On the basis of cytological examinations completed by simultaneous determinations of pregnandiol (Method of Huber, Borth and de Watteville) and estriol (Method of Brown) excretion, we could confirm the opinion generally admitted that an elevation of the Eosinophilic and Karyopyknotic Indices is the most frequent expression of a hormonal disturbance (Stamm 1-3). It is, nevertheless, impossible to determine exactly the nature of this hormonal disturbance in basing it on only one cytological examination. Thus, we could not observe cytological differences between threatened abortion with isolated estrogenic insufficiency and global luteo-estrogenic insufficiency. In order to determine the exact nature of the hormonal imbalance, we utilize the estrogen test, which consists of administering synthetic estrogens (Hexanoestrol) in increasing doses, according to the plan of Smith and Smith.

We have employed this test in 25 cases of threatened abortion and examined simultaneously the cytological modification and the behavior of the luteal function by repeated determinations of pregnandiol excretion. This study has led to the following conclusions which are summarized in Table I.

(1) A threatened abortion in which the pathological smear becomes normal under treatment with increasing doses of estrogens is not accompanied by either a serious luteal insufficiency, nor by a global insufficiency, but simply by an insufficient estrogenic stimulation (Fig. 1).

(2) A smear which becomes definitely worse under estrogenic treatment is pathognomonic of serious luteal insufficiency (Fig. 2). It is almost always a global luteo-estrogenic insufficiency, because isolated luteal insufficiency according to our experience seems rather rare (1 doubtful case in 35 threatened abortions examined).

Table I

Study of relations between cytological modifications and the hormonal situation in 25 cases of threatened abortion submitted to the estrogen test.

Smears at the onset of threatened abortion	Cytologic modifications under estrogen treatment according to Smith and Smith	Hormonal situation
25 cases of threatened abortion with pathological smears	17 cases, normalizing of indices in 20 days	18 cases, pregnandiol within normal limits (more than 50% of the norm)
	1 case, improvement of indices in 20 days.	
	7 cases, persistance of the elevated indices or aggravation of the smears in 20 days.	7 cases, with serious and prolonged luteal insufficiency (less than 50% of the norm)

II. Threatened abortions with normal or apparently normal smears

We have observed this cytological situation primarily in cases of threatened abortion complicated by isthmic insufficiency, myoma, placenta previa and pregnancies accompanied by serious toxemia.

In this group of threatened abortion we do not have a sufficient number of cases with simultaneous determinations of ovarian hormones to be statistically certain about our observations. Nevertheless, all cases to which the estrogen test has been applied and which were simultaneously explored by smears and hormonal determinations have given concordant results from which come the following conclusions:

Apparently typical pregnancy smears can react in three ways under the influence of increasing doses of estrogens.

(a) The apparently typical pregnancy smear is not modified by estrogenic stimulation.

This behavior has been seen in cases of normal luteo-estrogenic excretion. On clinical examination of four of these cases two isthmic insufficiencies were uncovered, one myoma undergoing necrobiosis and once a post-abortion status was seen.

We believe that this cytologic behavior is characteristic of threatened abortion without hormonal insufficiency.

MENACE D'AVORTEMENT AVEC INSUFFISANCE OESTROGENIQUE

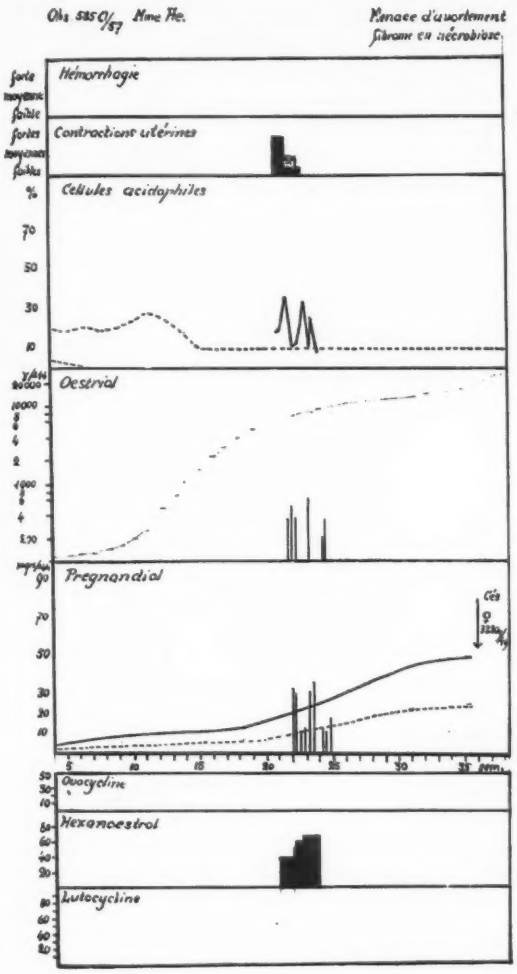

Fig. 1. Threatened abortion with Eosinophilic Index above 6. After treatment with increasing doses of estrogens (estrogen test), normalization of the vaginal smear. This is characteristic for threatened abortion accompanied by isolated estrogenic insufficiency.

MENACE D'AVORTEMENT AVEC INSUFFISANCE HORMONALE GLOBALE

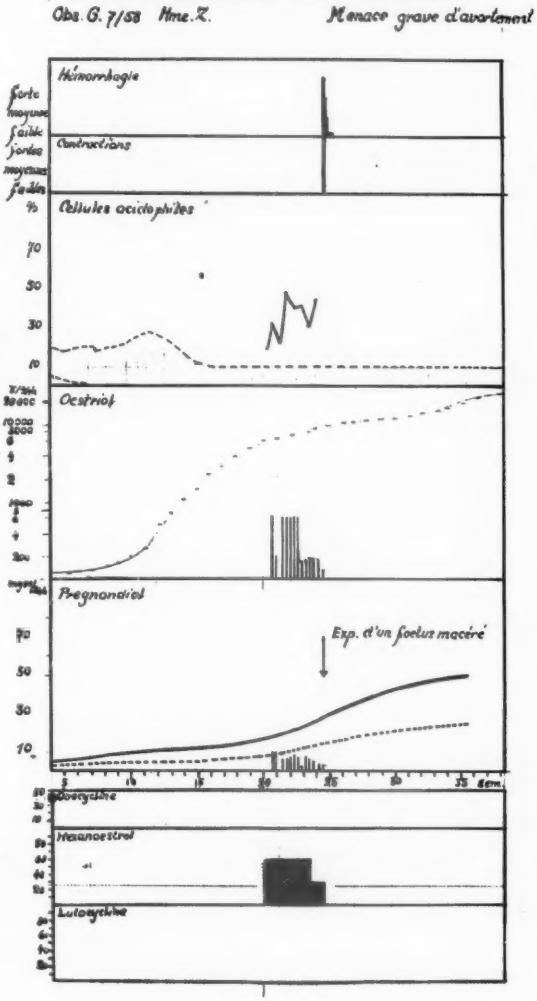

Fig. 2. Threatened abortion in which the vaginal smear definitely becomes worse during the course of the estrogen test. This behavior is pathognomonic of a severe luteal insufficiency. (The average excretion of pregnanediol in these cases is more than 50% below normal values). In most of these cases we are dealing with global luteo-estrogenic insufficiency.

(b) The apparently typical smear becomes definitely worse with increase of Eosinophilic and Karyopyknotic Indices under estrogen stimulation.

These cases present, according to our hormonal dosages, a serious and persisting luteo-estrogenic insufficiency. We observed this in three cases of toxemia and one case of eclampsia. We call these apparently normal smears pseudo-pregnant smears because genuine pregnant smears do not react with a definite increase in eosinophilia due to estrogenic stimulation.

(c) The apparently typical smear shows a temporary (10-20 day) eosinophilic increase under estrogenic stimulation.

This cytologic reaction is, according to our observations, the expression of a discrete and isolated estrogenic insufficiency.

SUMMARY

Simultaneous examination of vaginal smears and of the excretion of ovarian hormones in threatened abortions shows that an increase of Eosinophilic and Karyopyknotic Indices above the norm is generally accompanied by a hormonal disturbance. If the administration of synthetic estrogens according to the plan of Smith and Smith is followed by a normalization of the smear in about 20 days, the hormonal insufficiency is of the "estrogenic insufficiency" type.

In the case of persistance of pathological smears under estrogenic treatment, the hormonal insufficiency is of the global luteo-estrogenic type or eventually an isolated luteal insufficiency (rare-1 doubtful case in 35 cases examined).

In threatened abortions with apparently normal smears a hormonal disturbance is not very likely, if increasing doses of estrogens do not modify the smear. If, on the other hand, there is a persisting elevation of Eosinophilic and Karyopyknotic Indices, there exists a global ovarian insufficiency, which is most often severe and long lasting.

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DISCUSSION

MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil:

It is, at present, common knowledge that abnormally marked cellular eosinophilia and karyopyknosis in vaginal smears during pregnancy must be considered an early preclinical sign of imminent or threatened abortion, or premature labor. Rogers and co-workers (1) call it a "cytologically threatened abortion." Similar aspects have been, furthermore, observed in early and late toxemia of pregnancy (2), in premature separation of placenta (3) and in cases of maternal Rh-iso-immunization (4). This abnormal colpocytological pattern was also observed by us in three cases of diabetes (and pregnancy) and in two cases of invasive cervical carcinoma diagnosed close to term.

In principle and for practical purposes we accept the criteria proposed by Pundel and co-workers (5). However, we do not believe that the quantitative estimation of eosinophilic, karyopyknotic superficial cells can give a definite clue for the prognosis in cases of so-called "cytologically threatened abortion," or even in cases with clinical symptoms of abortion. We have seen many cases reaching a normal term in spite of exhibiting very high Eosinophilic and Karyopyknotic Indices; whereas in other instances patients with quite low indices developed clinically threatened abortion or actually aborted.

In our opinion the estimation of the desquamation rate (tendency of the cells to crowd together in clusters or agglutination phenomenon) is at least as important as the estimation of karyopyknosis and eosinophilia. The decrease or the disappearance of this "agglutination phenomenon," which goes along with a decrease of the Döderlein bacilli, regularly precedes abortion and premature labor, as well as, normal labor at term.

Relatively abnormally high amounts of eosinophilic, karyopyknotic superficial cells in pregnancy smears must be regarded as a non-specific sign of a disturbance of pregnancy. They may acquire, however, some prognostic specificity, as Pundel and co-workers first pointed out (5), when they fail to disappear after three or four days of administration of sufficient amounts of ovarian hormones. In our experience low dosages are useless. We give three to five mg. of ethinyl-estradiol by mouth and/or 100 to 200 mg of progesterone intramuscularly.

In the follow-up of patients treated with estrogens for "cytologically threatened abortion," there may be found four main patterns in our daily or weekly smear studies:

1) Persistence of the abnormal smear pattern three or four days after treatment.

2) Partial or total disappearance of the eosinophilic, karyopyknotic superficial cells and a return of the normal pregnancy pattern.

3) Appearance of a regressive colpocytological pattern resembling the cytological aspects of the genital crisis of the postpartum period.

4) Appearance of a marked, so-called "estrogenic" colpocytological pattern exhibiting up to 90% eosinophilic, karyopyknotic superficial cells.

Reactions 1) and 2) do not allow the drawing of definite conclusions as to the final outcome of the pregnancy, but it may be safely assumed that in 1) the prognosis is bad and that in 2) it is good.

Reactions 3) and 4) express two different colpocytological phases which mark and succeed the death of the ovum. In 3) the ovum is perishing or is already dead, and in 4) we have the genital recovery, in spite of a retained dead ovum, and the normal response of the vaginal epithelium as observed in the non-pregnant woman.

Reaction 4) is seen only in cases of pregnancies in the first trimester. It may be noteworthy to mention that we have observed markedly "estrogenic" vaginal smears in two cases of missed abortion, with presumed retention of the ovum for at least three months, in patients who did not receive any estrogens.

The appearance of a regressive colpocytological pattern in pregnancy (postpartum pattern) has been regarded as a sign of prenatal death of the ovum, and in general this is true. Nevertheless, one cannot rely too much on the significance of this cytological pattern. In clinically threatened abortion we have seen smears in cases which finally arrived normally at term. In such cases careful observation with an estrogen test should be made.

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HERBERT E. NIEBURGS, New York, New York, U.S.A.:

The participants in this symposium appear to agree on the general criteria for the cytological changes in abortion. An increase in the Karyopyknotic and Eosinophilic Indices appears to be suggestive of threatened abortion. The persistence or increase of the Karyopyknotic and Eosinophilic Indices is, during estrogen therapy, indicative of a poor prognosis. A substantial decrease in the Karyopyknotic and Eosinophilic Indices is a sign of continuation of normal pregnancy. The presence of parabasal cells, or so-called "postpartum cells," is an indication of death of the fetus. The finding of a normal vaginal smear, in threatened abortion, apparently is a sign of the non-hormonal cause of this disorder of pregnancy.

There appears to be some disagreement as to the amount of increase in the Eosinophilic and Karyopyknotic Indices. Dellepiane considers a Karyopyknotic Index below 35 and an Eosinophilic Index below 45 an indication of successful continuation of pregnancy. Clarice Ferreira quotes indices for eosinophilia and karyopyknosis as 10 for the border line for the evaluation of pregnancy disorders. Stamm, Rawyler and Riotton have found an Eosinophilic Index above 6 associated with threatened abortion. I cannot agree with Dellepiane that the persistence of the "pre-treatment" Karyopyknotic and Eosinophilic Indices is an indication of a good prognosis in cases of threatened abortion. It also seems that the degree of elevation of the Eosinophilic and Karyopyknotic Indices as stated by Dellepiane is far above that observed by other investigators. Cytolysis does not appear to be an indication of difficulties during pregnancy according to most authors. If abortion occurs with cytolysis, it is usually due to causes other than hormonal.

Most authors of this and related topics appear to be in agreement regarding the importance of the Eosinophilic Index in the evaluation of pregnancy disorders. It must be taken into consideration that the Eosinophilic Index is based on the staining reaction of cells, which is subject to many variables, such as the staining procedure utilized, the pH, vaginal infection, fixation, etc. In all papers presented on this subject a precise morphological description of the type of cells with eosinophilia is lacking. A more accurate evaluation might be possible if other standards such as cellular and nuclear dimensions would be taken into consideration.

The statistics presented by Stamm, Rawyler and Riotton on the incidence of pregnancies carried to term, in which disorders became evident by the use of the vaginal smear and treatment was instituted without delay, are certainly most convincing.

GUILLERMO TERZANO, Buenos Aires, Argentina:

During pregnancy, in the presence of a threatened abortion, any abnormal increase of the Eosinophilic Index should be considered as an indication of hormonal imbalance, while a normal Eosinophilic Index leads to the conclusion that the cause of the disorder is probably not of endocrinological origin.

It is worthwhile to remember the importance of cytological studies of urinary sediment in these cases, when bleeding may make an ordinary cytological evaluation difficult.

CLOSING REMARKS

GIUSEPPE DELLEPIANE:

In our main paper which was discussed by Terzano, Kamnitzer and Nieburgs, we pointed out the morphological features of vaginal cytology in threatened abortion during the first quarter of pregnancy, only touching on the most classical and evident changes, for the purpose of keeping our work within limits.

The data reported by Kamnitzer and Terzano agree with our point of view. It is, in fact, not sufficient to express a favorable or unfavorable prognosis of the pregnancy by the mere evaluation of the Eosinophilic and Karyopyknotic Indices. In fact, frequently, we have observed cases which have been checked periodically throughout the entire duration of the pregnancy. They presented values similar to the above mentioned data, e.g. up to 60; however, the development of the pregnancy was completely normal.

It is of significant value to note the behavior of the navicular, intermediate and basal cells and the modality of their exfoliation. Moreover, attention has to be paid to the cervical and endometrial also to elements of fetal origin.

On the basis of the case report examined, we deemed it advisable to establish the limits of the values of the Eosinophilic and Karyopyknotic Indices, in close connection with the other features of the smear since the indices permit a safer prognostic judgement, in the prognosis of pregnancy.

Therefore, we do not share the opinion expressed by Nieburgs, who considers an Eosinophilic Index higher than 10 decidedly unfavorable for prognosis, stressing what is mentioned above and what has been reported by a number of others (Van Meensel, Gaudefroy, Benson, Pundel, Kamnitzer, etc.).

Also, in regard to the behavior of the indices after administered hormones (ethinyl-estradiol and progestrone), we consider as a favorable prognostic sign a timely decrease of their values. We observe, however, that in many cases where said values remain stationary, pregnancy proceeds to term.

CLARICE DO AMARAL FERREIRA:

All speakers and discussants, if we look attentively, agree on the cytological patterns found in abortion. If sometimes they seem to disagree, it is only due to different ways of interpretation. Regarding the different Eosinophilic Indices which are considered by some authors as being normal during pregnancy, we should pay attention to the method of preparing the smears and especially the staining method used. This can modify the interpretation.

VAGINAL CYTOLOGY IN ECTOPIC PREGNANCY

ARTURO ANGEL ARRIGHI
Buenos Aires, Argentina

We had the opportunity to study the vaginal smears taken on 14 women before they underwent surgery for ectopic pregnancy. Four patients were amenorrheic and ten had metrorrhagia. The smears were obtained from seven days to one day prior to surgery. In the group of four amenorrheic patients the smears in three cases showed evidence of intense progestational stimulation characterized by folding of cellular edges, crowding of cells, Karyopyknotic Index lower than 30 and leukocytosis. The cytologic picture was suggestive of pregnancy. The smears of the fourth amenorrheic patient showed high Eosinophilic and Karyopyknotic Indices (50 and 65 respectively) and no evidence of progestational activity. It may be mentioned that in this particular patient the smears were obtained 24 hours prior to the episode of acute hemorrhage. In the group of ten patients with hemorrhage, three showed numerous erythrocytes in the smears, which precluded cytological evaluations. Three patients exhibited less numerous erythrocytes, but the smears showed various types of cellular constituents, and no sign of progestational effect.

A diagnosis of disturbed pregnancy was made in four patients because of strong progestational activity (low Karyopyknotic Index, folding of the cellular edges, crowding of cells, cytolysis, leukocytosis) with a variable number of red blood cells. The diagnosis of ectopic pregnancy was not made in any of these cases, but threatened abortion was suspected.

SUMMARY

Only in a few cases of ectopic pregnancy can valuable information be obtained by means of cytological smears. It can be assumed that the cytologic patterns of the smears change during the different stages of evolution of ectopic pregnancy.

DISCUSSION

J. ERNEST AYRE, Miami, Florida, U.S.A.:

Ectopic pregnancy provides a challenging study for the gynecological cytologist. Since both ectopic pregnancy and carcinoma in situ are frequently preceded by infection and inflammation, it is probable that long term statistical studies would demonstrate a higher incidence of carcinoma in situ in patients who have had ectopic pregnancy. We have not infrequently observed the association of precancerous or carcinoma in situ cell findings from the cervix in ectopic pregnancy. One of the interesting cytological manifestations, whether associated or not with atypical cells, during pregnancy is the rather consistent observation of a high Karyopyknotic Index in cervical smears or scrapings. Indeed, this would seem to be of diagnostic significance.

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

In several cases of suspected ectopic pregnancy (pain in the lower abdomen, vaginal bleeding after a period of amenorrhea, palpable mass in the region of the tubes) we have raised the question as to whether or not it is possible to differentiate between this condition and others such as ovarian cysts with menorrhagia or infections, by means of cytology.

Only five out of 18 cases of ectopic pregnancy revealed cells typical of pregnancy, i.e., of the navicular or cytolytic type. Three of these were cases of ruptured ectopic pregnancy. In all other cases we saw smears which were compatible with a "moderately estrogenic" or "slightly progestational" effect. In some the picture was not clear due to the presence of infection.

In cases of ovarian cysts with menorrhagia (follicle cysts) we repeatedly found a hyperfollicular cytological picture, which we did not see in our cases of ectopic pregnancy. In ectopic pregnancies we never saw a postpartum smear before the operation, even after long standing menorrhagia.

Vaginal flora consisting only of Bacillus vaginalis does not exclude an infective condition of the tubes.

JEAN A. de BRUX, Paris, France:

I fully agree with Arrighi's statement, but I should like to add several observations:

The process of ectopic pregnancy is not very well understood. Often the acute hemorrhagic episode occurs at the end of a rather long evolution period.

a) When a patient consults us because of a brief period of amenorrhea, generally the vaginal cytology is luteal or hyperluteal. The biological reactions are still negative, or so weakly positive that the pregnancy is very dubious. Cytologically, the ectopic pregnancy begins in the same way as does a uterine pregnancy.

b) The patient has a more or less abundant metrorrhagia, with or without expulsion of a uterine cast. The smear has a very high level of eosinophilic cells with pyknotic nuclei. This bleeding is the expression of the detachment of the ovum and of the expulsion of the embryo. The picture is the same as in a threatened abortion, but the site of the process is abnormal. If the patient has been followed since the beginning of the amenorrhea, abortion might have been suspected. Only laparoscopy permits the exact diagnosis.

c) Later, the question is even more difficult. After expulsion of the embryo, the decidua and the chorionic villi are still living, in the tube or in the tubal diverticula. They have a perforating action, sometimes very effective, since the tube is ruptured days and sometimes a few weeks after the expulsion of the embryo from the periovular membranes. The smears during this period are extremely variable, and they reflect the degree and variety of the hormonal action, of these membranes, of the corpus luteum (if it is still acting), and of the newly developing follicles. Hence, the smears are mostly impossible to interpret.

In view of these facts, it is impossible to designate smears of a precise type in an ectopic pregnancy.

It would be very important to follow up the smears from day to day when an ectopic pregnancy is suspected.

Inversely, during this period the endometrial biopsy permits the diagnosis of interrupted pregnancy, uterine or extra-uterine. In fact, Arias Stella has described atypias at the site of the glandular tubes, and we ourselves together with Vaissade have described anomalies of the stroma. In this way we may determine if the pregnancy has been interrupted. In more than 60 cases we have correctly made the diagnosis of extra-uterine pregnancy.

CLOSING REMARKS

ARTURO ANGEL ARRIGHI:

As the discussants agree, the cytologic picture of the ectopic pregnancy varies with the evolution of the disease.

DIAGNOSIS OF PREGNANCY BY MEANS OF CYTOLOGY

B. CORNELIS HOPMAN
Miami, Florida, U.S.A.

The cytologic diagnosis of pregnancy has not been successful up to now. The causes of failure are the too many variables in the microscopic field. Cytolysis may or may not be present. Free nuclei and cell fragments may or may not be visible. Karyopyknosis, eosinophilia, cyanophilia, number of cells, oyster shell cells of Papanicolaou, all may be present in such different degree that general rules for the diagnosis are difficult to make. It seems, further, that the hormonal picture in pregnancy is so variable that estrogen and progesterone levels also seem to change in different degrees.

In 1950 attempts were made (1, 2) to bring some order into these variables and put together a general outline in the cytologic observations. On the basis of the hormonal changes as recorded in Riley's (3) "Essentials of Gynecologic Endocrinology," a test was made to understand the cytologic observations in relation to the hormonal changes. Four stages of pregnancy were distinguished. Up to the third month of gestation intact vaginal epithelia were seen, for the most part basophilic with intact vesicular nuclei and greyish-blue cytoplasm. This was probably under the influence of the rising chorionic gonadotrophin production, although not generally acknowledged. No cytolysis with cell fragments or free nuclei was observed. Estrogen reaction of the epithelia was low. From the third till the sixth month, when the gonadotrophin values are decreasing, an increasing cytolysis with cell fragments and free nuclei together with increasing Döderlein bacilli was observed. After six months an increasing amount of estrogen re-established an intact cell picture with decreasing cytolysis and more pyknotic nuclei. At the end of pregnancy a cell picture arose resembling that of a newborn female child: intact cyanophilic cells with vesicular or rod-shaped nuclei, often with thickened cell borders. Now and then the cells joined together by bridge-like processions.

Women past 40 years show cytolysis with cell fragments, free nuclei and Döderlein bacilli as a normal physiologic appearance independent of pregnancy. Furthermore, the nucleus of the cyanophilic cells may show the exact pregnancy structure with condensation of the chromatin along the nucleus like the veins of a leaf (1). Though there are basic differences between the cells after age 40 and the cells of pregnancy, we decided to concentrate our efforts on tabulating the pregnancy characteristics of vaginal smears of non-postnatal women 40 years or younger. This age restriction does not seem too serious, since the ages of 100 consecutive pregnant women in our laboratory were 35 years or younger - 95, 36 years - 3, 37 years - 1, and 38 years - 1.

DIAGNOSTIC CRITERIA

Ten criteria are distinguished as follows:

1. General cell population of the slide. The cells seen in slides of pregnant women are more cyanophilic than in non-pregnant women. The ratio of cyanophilia to eosinophilia is generally four or six to one. This rule is not without exceptions. Under the influence of infections, especially Trichomonas, an eosinophilic reaction may prevail together with a poor-staining appearance of the nucleus, nuclear shrinkage and halo formation. It is, therefore, important to take infections into account if an eosinophilic cell picture prevails. This, then, is not a criterion against pregnancy. Furthermore, cells grouped in clusters may have an eosinophilic appearance although they are really cyanophilic, as proven by the cells lying at the periphery of the clusters.

The cells during pregnancy are for the greatest part intermediate cells, because this cell layer shows the most hypertrophy during pregnancy. Because superficial and basal cells are in the minority, the

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cells in pregnancy are mostly of equal size and smaller than the superficial epithelia. Differences of color and cell size are displayed definitely less frequently than in a smear from a non-pregnant patient. Although the cells form groups in close proximity, overlapping occurs less frequently than in non-pregnancy. Cells in close proximity often show a more or less pronounced cell border, which is lacking in slides of non-pregnancy. Other cell types such as small basal cells and endocervical cells often seen in non-pregnancy are uncommon in slides of pregnancy.

2. Papanicolaou [4] as early as 1943 described in his book "Diagnosis of Uterine Cancer by the Vaginal Smear" a cell in pregnancy of the oyster shell type. It was an intermediate "navicular" cell with dense cytoplasm and an elongeated nucleus showing dark borders mostly thickened and appearing in greater numbers in pregnancy. They are more apparent when cells appear in groups than when single. This cell, often seen in slides of pregnant women, is, however, not constantly present and may also resemble cells in women past 40 years of age nearing the climateric and in secondary amenorrhea.

3. Döderlein bacilli occur very often in pregnancy. They are one of the most constant findings and are of great diagnostic value. Under the influence of cytolysis, glycogen is formed in greater quantities than normal, probably forming the foodstuff in which the organisms multiply. Though constant in pregnancy they occur also in slides of women nearing or past the menopause, reasons why we excluded these groups from our diagnostic efforts.

4. Cytolysis is a very important occurrence in pregnancy. Though not as continuous as Döderlein bacilli, we find it in about 30% of the pregnancy cases. It presents itself as cell fragments, bluish ill-formed cytoplasmic formations of indistinct size and shape. The blue color is often faded and the fragments may be confused with Trichomonas vaginalis.

5. Free nuclei appear when cytolysis has resulted in total degeneration of the cell, leaving cell fragments and free nuclei more or less attached to some cytoplasm. As a contrast to the degenerated cytoplasmic fragments the naked nucleus still has an intact appearance with oval form, intact nuclear border and leaflike chromatin condensations.

6. Besides the Papanicolaou oyster shell cells, others exist which are characteristic of pregnancy. They are round or oval cells of light blue color with dark, but not thickened, borders and narrow elongated nuclei. The latter may lie parallel to the cytoplasmic border but often are perpendicular to it. We find these cells almost as often as the oyster shell cells and they are of great diagnostic significance. Oyster cells are mostly described as navicular cells. Now and then the same kind of cells, with dark enlarged borders and dense blue cytoplasm, are found to be almost round.

7. Cells appearing in pregnancy in close proximity often have a furrowed appearance. Probably because of lack of space these cells wrinkle and the blue cytoplasm appears as streaks and stripes along the cell body.

8. In pregnancy the cytoplasm of large cyanophilic cells with vesicular nuclei is mostly darker blue and has a more dense appearance than in non-pregnancy. The cytoplasm of eosinophilic, karyopyknotic superficial cells in pregnancy also is more distinct and well stained than in non-pregnancy.

9. Now and then the cytoplasm of cells in pregnancy shows a yellow hue probably caused by an increased glycogen content.

10. The nuclei of pregnancy cells are very characteristic and have the greatest diagnostic importance. The pyknotic nuclei of the eosinophilic cells have a dark, almost black, appearance. They are seldom round, mostly oval, and have a dark nuclear border. The chromatin shows fine dark granulations and appears active. There is a distinct difference between the pyknotic nucleus of a squamous superficial cell in the non-pregnant and in the pregnant state. In non-pregnancy the nucleus is often round, dull-looking, inactive, often reddish instead of dark and, though larger than one segment of a polymorph, is of comparable size. In pregnancy the nucleus is of distinctly larger size, mostly two or three times the size of one segment of a polymorph or larger. The nuclear membrane of a pyknotic nucleus in non-pregnancy is often dull, non-conspicious; the nuclear membrane in pregnancy is distinct, dark and conspicious. Anucleated squamous cells are seen now and then in pregnancy.

More important, however, are the nuclear characteristics of the large cyanophilic cells with vesicular nuclei. We consider them as very constant, almost always present, easily recognizable and very specific. They have a nucleus with granular chromatin coarser than the pyknotic nucleus. The nucleus looks very active, is elongated and narrow or oval, seldom round. It has a distinct nuclear membrane. In the center of the nucleus is a linear condensation of chromatin like the central veins of a leaf. From it branchings and ramifications of chromatin condensations run to the nuclear membrane. These nuclei were first described in 1950 [1]. Originally these lines were taken for Döderlein bacilli covering the nuclei, but seem to be chromatin condensations or concentrations for they also occur when bacilli are not present. There is a striking difference between the nuclei of large cyanophilic cells with vesicular nuclei in pregnancy and non-pregnancy. In non-pregnancy the nuclei often show no chromatin activity, no granulation, no chromatin concentrations; instead of a dark color, often a light one is present; they may even appear as a shadow. The size is distinctly smaller. They are more round or oval rather than elongated.

Although none of the mentioned criteria are foolproof characteristics of pregnancy, together they form a sound foundation for pregnancy diagnosis.

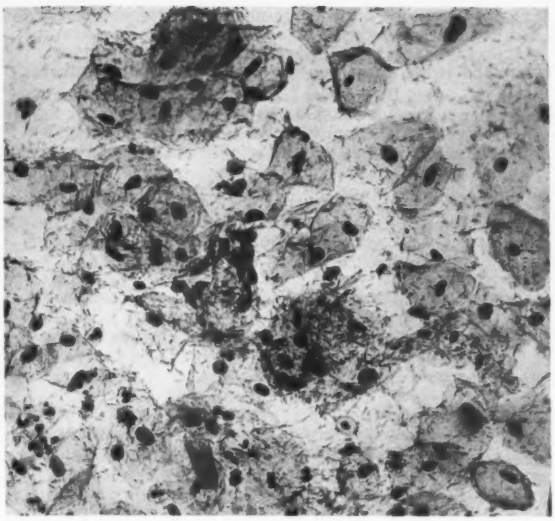

Fig. 1. Cells of vaginal smear of 6 months pregnancy. Eosinophilia, cells of uniform size. Oyster shell-type of cells upper left. Döderlein bacilli scattered around. Free nuclei in the lower center. Nuclei of Karyopyknotic cells (upper center) are distinctly larger than a segment of a polymorph (bottom left). Vesicular nuclei are elongated or oval, seldom round, and show leaflike chromatin condensations.

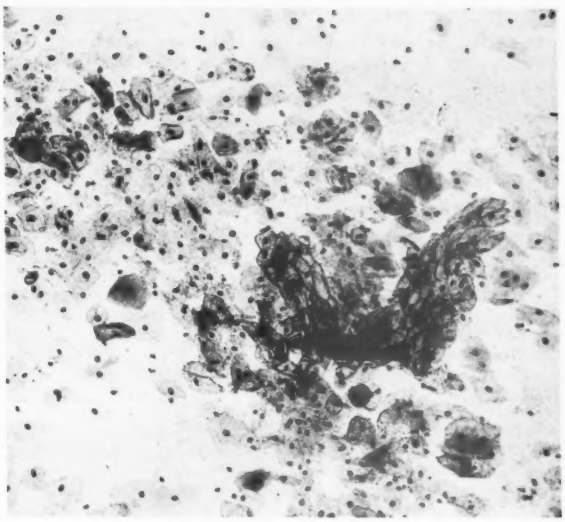

Fig. 2. Cells of vaginal smear of 6 months pregnancy. Above and under the V form of oyster shell cells there are some round cells with darker borders and elongated nuclei perpendicular to the cell border. Indistinct cell fragments and free nuclei scattered around.

DISCUSSION

The use of the vaginal smears in diagnosing pregnancy was already advocated by Papanicolaou in 1925[5]. In 1943 he described the oyster shell navicular-type of pregnancy cell[4]. In 1948 Papanicolaou described cells seen in catheterized urine of the same character as the navicular cells and stressed the significance of urine as a basis for pregnancy diagnosis because it shows a simpler and more uniform cytology than the vaginal smear[6]. It is also much less contaminated with bacteria, leukocytes and histiocytes. Its practical value as a routine diagnostic procedure, however, was still to be determined.

Research done by Enrique del Castillo, et. al., in 1949, showed that the cells of the lower two-thirds of the urethra are lined with stratified squamous epithelium similar to the vaginal epithelium[7]. These cells undergo the same changes under hormonal stimulation as does the vaginal epithelium. This is probably due to the embryological fact that both vagina and lower urethra originate from the urogenital sinus[8,9].

This was followed by efforts in 1950[2] to try to diagnose pregnancy using the urethral smear. The smear was made with a sterilized round wooden stick, 13.5 cm x 0.25 cm, with a smooth finish. Without any harm this rod was introduced one to two cm into the urethra where some mucus was obtained,

smeared on a slide and stained by the Papanicolaou method. Three hundred preparations showed most of the aforementioned pregnancy signs. The smears had generally displayed a normal cyanophilic staining reaction without hindering eosinophilia caused by infections, a normal nuclear stain and no interfering Trichomonas, leukocytes or erythrocytes. The cells were of the same character as the vaginal epithelia and displayed similar pregnancy criteria.

Repeating the investigation with vaginal smears, our diagnostic results in the first hundred slides showed 89, in the second hundred 91, in the third hundred 92 in accordance with clinical diagnosis.

False-negative results are caused by infection, especially Trichmonas, causing a covering of the cells with leukocytes, causing eosinophilia, nuclear shrinkage with halo formation and loss of nuclear stains, the most important of the pregnancy characteristics. Dyskaryosis and cancer are also causes of false-negative diagnosis by the resulting eosinophilia and nuclear distortion. False-positive results are caused by infection and by some secondary amenorrhea cases, showing cells resembling the Papanicolaou oyster shell cell and cells with pronounced nuclear activity, chromatin condensation and ramification.

Vaginal smears combined with urethral smears give better results than vaginal smears alone.

CONCLUSION

A cytologic diagnosis of pregnancy by vaginal smears has not been successful up to now. Vaginal smears are not satisfactory to diagnose pregnancy, because infections, especially Trichomonas vaginalis, cause eosinophilia, covering of the cells with leukocytes, fading of the nuclear stain and shrinkage of the nucleus with halo formations. A vaginal smear combined with an urethral smear is more suitable for pregnancy diagnosis than a vaginal smear alone. The cellular characteristics for pregnancy are similar. Ten diagnostic criteria are mentioned. In diagnosing pregnancy the nuclear characteristics of the cells are of greatest importance. The pyknotic nuclei are larger, more active and have darker nuclear borders than in non-pregnant women. A segment of a leukocyte can be taken as a comparison. In large cyanophilic cells the vesicular nucleus is stretched or oval, seldom round, and shows chromatin condensations like the veins of a leaf. None of the mentioned criteria is a certain proof of pregnancy, but together they form a sound basis for differentiation which combined with an urethral smear brings the diagnosis of pregnancy by cytology very near to a solution.

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DISCUSSION

MARCEL GAUDEFROY, Lille, Nord, France:

I would like to congratulate Hopman for his attempts to find significant criteria for the cytological diagnosis of pregnancy. However, it is important to say that the various cytological features studied are far from being diagnostic, separately or jointly considered.

Above all, the difference between the occurrence of pyknosis during pregnancy and the occurrence of pyknosis in smears of non-pregnant women does not seem clear to me. If the pyknotic nucleus is defined as a nucleus with condensed and non-structural chromatin and with a diameter smaller than 6μ, the cells in the upper center in Figure 1 are really not pyknotic but merely pre-pyknotic.

Therefore, I should like to suggest the use of a very simple test of pregnancy: It is based on the response or lack of response of the vaginal epithelium to administered estrogens. In amenorrhea of non-pregnant women with 10 mg daily of diethylstilbestrol (or 200 gammas of ethinyl-estradiol) for the period of five days, the Eosinophilic and Karyopyknotic Indices of vaginal cells increase immediately, and the cell type becomes "estrogenic." In normal pregnancy, however, the smear pattern does not change, and the very low percentage of superficial cells does not increase: the Karyopyknotic Index remains under 5, and the Eosinophilic Index under 10.

FRANTIŠEK HORÁLEK and MOJMÍR SONEK, Brno, Czechoslovakia:

We agree with Hopman that a direct cytological diagnosis of pregnancy is very difficult. In spite of the fact that the author gives a survey of diagnostic criteria, we believe that the diagnosis is more often a result of the total sum of all these criteria and especially a result of the general impression of the specimen. This circumstance, as well as the fact that some criteria of Hopman are not specific for the smears of pregnancy only (paragraphs 3, 4, 5), lead to the conclusion that direct cytological diagnosis is very unreliable for a less experienced cytologist.

Therefore, we resorted to an indirect diagnosis of pregnancy, based on the fact that the cytological picture during pregnancy does not react to the application of estrogens. This fact has been mentioned several times in the articles in this issue concerning the effect of administered estrogens. Our findings are based on 85 cases of negative estrogenic effect in pregnant women in the period between the 4th to 12th week of pregnancy. We applied diethylstilbestrol in doses of 5 - 15 mg. and estradiolbenzoate 5 - 10 mg., administered for 3 days; we have never found an estrogenic effect.

It is questionable whether or not this reaction is strictly specific for pregnancy. Negative estrogenic reaction can occasionally occur in (1) corpus luteum persistence, (2) hydatiform mole, chorionepithelioma, and (3) intact extrauterine pregnancy. In view of the small number of cases under observation we cannot form a conclusive opinion about these cases.

This indirect cytological test has been proven to be a very reliable aid, especially for differentiating pregnancy from certain common states, which cause uncertainty in the diagnosis (myoma, hypohormonal oligomenorrhea and amenorrhea, etc.).

MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil:

In the earlier literature on colpocytology in the diagnosis of pregnancy, Cohen and Rubenstein (1) reported an accuracy of 84% of correct diagnoses in 200 cases, and Medina and Silva (2) reported that colpocytology is not a reliable method for the diagnosis of pregnancy.

It is true that one often finds typical smear patterns in cases of recent amenorrhea, so that one is tempted to make a diagnostic statement. At other times the colpocytological pattern is less typical and subject to doubts. But since there is no such thing as a "slightly pregnant" or a "very pregnant" woman, it must be concluded that a definite diagnosis of pregnancy by colpocytology is out of question.

There remains, however, a possibility which has not been sufficiently explored. The peculiar lack of response of the vaginal epithelium to administered estrogens during pregnancy may possibly be used as a diagnostic test, as Wied (3) has recently shown.

A test using the crystallization of the cervical mucus was previously developed by Zondek and Cooper (4). Rodrigues Lima and others (5) were able to reduce substantially the time required for this test by the intravenous use of estrogens. These tests are most interesting from a speculative point of view, but they are of little practical value.

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J. PAUL PUNDEL, Luxembourg, Luxembourg:

Dr. Hopman's paper merits many comments, but I prefer to discuss only the main problems of the practical value of the vaginal smear as a possible cytological test for pregnancy, problems which have not been presented by Hopman.

First, I have to make three important objections: Hopman has not specified for his results:

1. at which moment of the pregnancy the smears have been taken,
2. if the pregnancies have been normal or not.
3. a counter-experiment of vaginal smears in non-pregnant women in order to find out the possible frequency of false-positive diagnoses.

These three points are essential for the study of the vaginal smear as a pregnancy test. In 1951 Van Meensel and I presented a rather long discussion about this problem (1), in which Hopman can find the details of the diagnostic difficulties and the practical problem for the clinician. For this symposium I would like to present only a summary of my present experience:
(1) There does not exist any particular cytological modification of the vaginal smear which could be considered as specific for pregnancy. Even the particular type of nuclei described by Hopman can be found frequently in non-pregnant women.
(2) The vaginal cytology reflects only the activity of the sexual hormones such as estrogens, progesterone and androgens, but no specific affect of chorionic gonadotropins as the usual biological pregnancy

tests. Pregnancy smears can be artifically produced or observed spontaneously, if there exists a marked activity of estrogens and progesterone or if these two hormones are injected or secreted in the right proportions. Such hormonal activity can be observed under hormonal treatment and in some menstrual disorders without pregnancy.
(3). The typical pregnancy smear can be observed only in normal pregnancies and generally only after the end of the third month, that is, at a moment when a trained obstetrician in most cases can make a correct diagnosis by clinical examination.
(4) In abnormal pregnancies the vaginal smear can present a pure estrogenic smear pattern in which nothing permits a cytological suspicion of pregnancy.

For practical purposes a pregnancy test should give correct results at the beginning of pregnancy and in cases with difficulties in making the clinical diagnosis, especially with abnormal bleeding. A pregnancy test which can give a maximum of accuracy only after the third month of pregnancy and only in normal pregnancies represents no particular value to the clinician.

I have examined for this discussion the vaginal cytological findings of over 3,000 cases of early pregnancy and amenorrheas or menopauses or cases where pregnancy could be suspected. In 1,240 cases of pregnancy during the first three months, a positive diagnosis of pregnancy by vaginal smears could be done in only 72% of these patients. In the remaining 28% a positive cytological diagnosis of pregnancy was not possible, because the vaginal cytology had not yet presented the typical pregnancy pattern or because hormonal disturbances existed which produced abnormal smears.

In 1,800 non-pregnant patients who consulted us for amenorrhea, including patients suspecting pregnancy at the beginning of the menopause, the vaginal smear showed cytological pictures identical to that of pregnancy in 9%. If the patients with cytolytic smears are added, where no differential diagnosis is possible between pregnancy and amenorrhea or menopause, the percentage of dubious or false positive results would be over 15%.

I think that these results are sufficiently demonstrative to permit the conclusion that the vaginal smear cannot be used as a reliable pregnancy test. But this does not reduce the important practical value of the vaginal smear for the control of the hormonal conditions of pregnancy: If the diagnosis of pregnancy is established and if the vaginal smear presents a corresponding normal pattern, one can conclude that the pregnancy has a normal hormonal level. If, on the other hand, in a pregnant patient the vaginal smear shows a different pattern, this can be accepted as a very reliable evidence of a hormonal disorder which, if untreated, may result in spontaneous abortion.

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CLOSING REMARKS

B. CORNELIS HOPMAN:

To J. Paul Pundel:

I thank Pundel for his detailed discussion of the problems of the vaginal smear for pregnancy diagnosis. First I want to remark that our aims have been different. Pundel thinks of cytology of pregnancy as a test in early pregnancy. But the subject "Diagnosis of Pregnancy by Means of Cytology" (in this Symposium) in my opinion indicates the investigation of diagnostic criteria of pregnancy at any stage. If and when these criteria have been met, then, as a second point, the value of the test in early pregnancy must be determined.

About lack of specification of the results I may answer:

1. In the subject there was no restriction as to the moment of pregnancy the smear was to be taken.
2. The subject contained no restriction for normal or abnormal pregnancy.
3. Slides of all non-pregnant women examined in the cytologic laboratory served as counter-experiment.

The paper gave a description of the frequency of false positive and false negative cases and their possible causes. During last year, pregnancy was diagnosed in 2500 cases out of 6000.

(1). I agree with Pundel that there exist no particular cytological modification of the vaginal smear specific for pregnancy, as I stressed in "Diagnostic Critera" and later repeated in the conclusion, but the same is true in cancer.

(2). I believe that the endocrinology of pregnancy is still a complex field. Many hormones are elevated - not only estrogens, progesterone, and chorionic gonadotropin hormones, but also relaxin, glucocorticoids and aldosterone. The influence that these and other hormones and their combinations have on the cell structure is only vaguely known. I have never succeeded in changing the cell picture to a typical pregnancy type of smear by injecting estrogens and progesterone or combinations of them.

(3). The typical pregnancy smear was found in cases where the gynecologist diagnosed or expected pregnancy

on the basis of anamnesis or his clinical examination. There was no restriction in time of pregnancy. The earliest pregnancy cases were fewer, because the clinical diagnosis was not known in most cases and often could not be followed in a population which frequently moves. However, in several cases we could diagnose pregnancy before the next missed period. Cases from the second until the ninth month were evenly divided in the reported cases. In these cases there was no difference found in exactness of diagnosis.

(4). Most of the cases where I found a pure estrogenic smear in pregnancy were soon followed by abortion. I did not add these to my false negatives, because they should be called false clinical positives not false cytologic negatives. Many other estrogenic smears were caused by infection and form a great part of my false diagnoses.

Pundel found a positive cytologic diagnosis in 72% of 1240 cases of pregnancy during the first three months. This gives much hope for the future. I believe our results in cancer diagnosis are not much better. In my thesis (1) I dealt extensively with the results in cancer diagnosis. The false positive and negative diagnoses calculated against non-cancer cases amount to only a few per cent, but calculated against cancer cases they amount up to 25 per cent. The same is true with pregnancy diagnoses. Counted against non-pregnant women, the results are as I described, but calculated against a specific difficult group of early pregnancies with hormonal disturbances, they are much worse of course. I described false positive diagnoses in amenorrhea. The only restriction I made was an age of 40 years, because women beyond that age and climacteric and menopausal patients may normally display cytolysis, Döderlein bacilli and cell structures resembling pregnancy. They practically did not change the results because in our population groups pregnancy above 40 years does not appear in statistically worthwhile numbers.

From the second till the ninth month false results were observed more as a result of infection, especially Trichomonas (causing false eosinophilia and nuclear shrinkage), than as a result of the period of pregnancy. Recent experiments with irrigations of acetic acid, lactic acid, vaginal installations of sulfa drugs and penicillin to clean the vagina have improved the results considerably.

I must apologize to Pundel and Van Meensel for not mentioning their studies in the references. In Europe, American literature is readily available. In the United States, European literature does not reach us so easily.

To Mario de Benning-Kamnitzer:

In most cases the diagnosis of pregnancy by cytology is possible and reliable in up to 90% of the cases, and by vaginal irrigations and installations the results will be improved.

This investigation is not to diagnose pregnancy exclusively in the earliest period. The cytologic diagnosis of pregnancy is important in any stage of pregnancy, not only to make the differential diagnosis of pregnancy against other deviations, but also to evaluate the prognosis of pregnancy. I believe the experiments of Wied with administered estrogen during pregnancy have scientific value as well as the crystalization test of the cervical mucus by Zondek and Cooper.

To Marcel Gaudefroy:

In pregnancy as well as non-pregnancy there are cells with pyknotic nuclei, although in pregnancy they are much less frequent. The difference is that the pyknotic nucleus in pregnancy is darker, contains more chromatin, is coarser, larger and has a more marked nuclear border. Comparison may be made with a leucocyte.

To František Horálek and Mojmír Sonek:

I believe that not only criteria 3, 4 and 5 but all of them, especially 10, are of importance for pregnancy diagnosis. As already stated in the closing remarks to Mario de Benning-Kamnitzer, administration of estrogens during pregnancy has scientific value. I did not use it extensively enough to be able to estimate its practical value.

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URINARY CYTOLOGY DURING PREGNANCY

ARTURO ANGEL ARRIGHI AND GUILLERMO TERZANO
Buenos Aires, Argentina

Urinary smears of pregnant women containing superficial squamous epithelial cells, both eosinophilic and cyanophilic, and intermediate cells, are in some respects similar to vaginal smears.

During the first three months of normal pregnancy the cytological picture is rather unstable and a definite pattern cannot be given. This period, called the "ovarian phase," is characterized by remarkable variations in the Eosinophilic Index and in the Karyopyknotic Index. Sporadic increasing of the eosinophilic cells may be observed, but a progressive and steady lowering of the Eosinophilic and Karyopyknotic Indices is more common.

From the end of the third month until the last month of normal pregnancy the cytology of urinary smears (as in vaginal smears) is more stable and uniform, with an Eosinophilic Index between 2 and 10 and a Karyopyknotic Index usually below 20. Many of the intermediate cells look like the navicular cells described by Papanicolaou, but being less frequently found in urinary smears than in vaginal smears, these cells appear more often isolated than in groups.

Although cytology of the sediment of voided urine nearly corresponds to the cytology of vaginal aspirations, urinary smears are often scanty and insufficient for a correct diagnosis; also, compared with vaginal smears, it is more difficult to assess progestational effect. Nevertheless, we accept urinary smears of pregnant women. Threatened abortion is the principal reason for asking for this test. Under such circumstances bleeding makes it difficult to evaluate vaginal cytology, and patients being afraid of any vaginal examination refuse even a vaginal aspiration. Some women also prefer, for one reason or other, to send urine to the laboratory rather than go to the clinic, especially when bed rest has been recommended.

The presence of vaginal infection, irritations, or other possible causes of false eosinophilia, may be an indication for urinary smears.

In women with threatened abortion due to hormonal cause, daily observation of urinary smears provides good signs:

1. To establish the existence of hormonal cause of the abortion.
2. To follow up the process.
3. To make a prognosis of the case.
4. To observe the effect of the prescribed hormonal therapy on the patient.

MARIO GONZALEZ RAMOS
Mexico, D.F., Mexico

Our experience is based on more than 6,000 cytological studies, processed with our own technique (1, 5) and using Papanicolaou's classical stain with a slight modification which was essential in order to obtain an adequate color for the cells of the urinary sediment (Figs. 21, 22).

The term "urocytogram," proposed by the Argentine authors (4, 9), is used and defined as: "the quantitative and differential cytological study of the urinary sediment." In carrying out this study we counted from 300 to 400 cells in different fields, also taking into account the general aspect of the smear.

I. NORMAL PREGNANCY

A. During the first weeks of pregnancy, and sometimes before the biological reactions for an early diagnosis of pregnancy appear positive, we find a cytological image quire different from that of the luteal phase it replaces. There are:

1. A few superficial cells, mostly blue with vesicular nuclei; not more than 10% eosinophilic, karyopyknotic superficial cells.
2. Abundant intermediate cells; more than 60%, some of which have eccentric nuclei. Some of these cells are "navicular" or "oyster cells."
3. Basal cells; up to 15%.

General aspect of the smear: clear; agglutination of both the superficial and the intermediate cells; the cytoplasm is not folded; few leukocytes; all of the cells exhibit vivid colors.

B. From the fourth to the eighth or ninth week of pregnancy the above characteristics are more apparent:

1. The superficial cells decrease in number; there are not more than 15%, of which 5% are eosinophilic, karyopyknotic superficial cells.
2. The intermediate cells increase, and clusters of "navicular" and "oyster cells" appear.
3. The basal cells also decrease in number.

General aspect of the smear: clear; with an abundance of cells; few leukocytes.

C. Between the tenth and twelfth week, and sometimes between the ninth and fourteenth week, we find:

1. Abundant superficial cells, 30% or more; the eosinophilic, karyopyknotic superficial cells increase up to 20%.
2. The clusters of intermediate cells have disappeared, and there are few "navicular" or "oyster cells."
3. Very few basal cells, not more than 5%.

General aspect of the smear; "dirty": there is an increase in the number of leukocytes, slight or moderate cytolysis; the cellular agglutination is either slight or has disappeared. The aspect does not seem to be that usually found in a pregnancy.

D. From the 14th to the 38th week there is a definite cytological picture that we call "gestational picture with normal steroid activity" or "definitive pregnancy pattern."

1. Very few superficial cells, not more than 5%; eosinophilic, karyopyknotic superficial cells from 0 to 2%.
2. An abundance of intermediate cells; numerous clusters and a great number of "navicular" or "oyster cells."
3. Basal cells have disappeared or are very scarce.

General aspect of the smear: clear, with an abundance of cells and marked cellular agglutination. Practically all of the cells are blue.

E. Final stage of pregnancy. Towards the end of pregnancy the cytological picture we have just described reverts to smear pattern exhibited shortly prior to term, having characteristics we have considered as "signs of placental senility" (1, 2).

1. The superficial cells increase in number up to more than 20%; eosinophilic, karyopyknotic superficial cells increase from two to ten per cent or more.
2. The clusters of intermediate cells decrease.
3. The basal cells increase to a variable degree, from five to twenty per cent.

General aspect of the smear: clear or slightly "dirty." Scarce leukocytes, cytolysis to a variable degree. Cell dispersion.

F. Labor. After the artificial rupture of the fetal membranes has taken place in induced labor or during spontaneous labor, there appears a cytological image which is entirely different from all of the others described.

1. The superficial cells show signs of cytolysis and are scarce. Some of them are multinucleated.
2. The intermediate cells are dispersed and show two to six or more nuclei.

3. There is an abundance of basal cells, more than 20%; practically all of which are binucleated.

General aspect of the smear: "dirty," oligocellular, slight agglutination, especially of the basal cells, marked cytolysis, leukocytes, histiocytes and red blood cells.

G. Puerperium. Twenty-four to forty-eight hours after childbirth oligocellular exfoliation is found. Almost all of the cells are basal, with a tendency towards eosinophilia. Abundant histiocytes, leukocytes and red blood cells.

II. PATHOLOGICAL PREGNANCY

A. Threatened abortion. There are four different cytological aspects (2, 3):

1. The most frequent (81%) which we called progestational deficiency characterized by:
   a. An abundance of superficial cells, more than 40%; karyopyknotic superficial cells more than 30%; eosinophilic, karyopyknotic superficial cells more than 10%.
   b. Intermediate cells: slightly agglutinated. Scarce "navicular" and "oyster cells." Some eosinophilic cells.
   c. Basal cells: either few or not found at all.

General aspect of the smear: clear or slightly "dirty." Variable degrees of cellular dispersion. Occasionally there is slight cytolysis; scarce leukocytes.

2. Aspect of mixed steroid (estrogen-progesterone) deficiency characterized by a slight or severe atrophic aspect.
   a. Superficial cells, very scarce or not found at all.
   b. Intermediate cells, slightly agglutinated and very few "navicular" or "oyster cells."
   c. Basal cells, to variable degrees from 20 to 60% or more, and in clusters.

General aspect of smear: "dirty," marked cytolysis; an abundance of leukocytes and histiocytes. Dispersion of intermediate cells.

3. Aspect of estrogenic deficiency; we have found this in 4 to 5% of our cases. It is characterized by:
   a. Scarce superficial cells 10 to 20%. Some eosinophilic, karyopyknotic superficial cells.
   b. Agglutinated intermediate cells. Few "navicular" or "oyster cells."
   c. Abundant basal cells, more than 20%, some of them eosinophilic.

General aspects of the smear: clear; only the large number of basal cells and the fact that some of them are eosinophilic is surprising.

4. The cellular pattern of normal pregnancy already described as "gestational picture with normal steroid activity."

It is important to point our that each cytological image is interpreted according to the stage of pregnancy, in relation to the different aspects of a normal pregnancy.

In the habitual aborter we may find any one of the four aspects described above.

B. Fetal death: Severe atrophic aspect characterized by: "Dirty" smear, with more than 50% basal cells, considerable cytolysis of the intermediate cells, abundant leukocytes and histiocytes.

These atrophic features do not appear if estrogens and/or progesterone have been administered.

C. Extra-uterine pregnancy: The cases studied (2, 10) with a dead fetus exhibit the atrophic features just described.

D. Incomplete abortion: Atrophic cellular features.

E. Threatened premature labor: We find the image indicated as the final one in normal pregnancy, except for the fact that there is noticeable cytolysis present, and an increase of more than 20% basal cells can be found.

Clinical control of any possible abortion and observation of habitual aborters may be obtained by means of periodic urocytograms which show whether or not there is any need for the administration of hormonal therapy (3, 6).

F. Hyperemesis gravidarum: In all the cases studied, there have been found three different cytological patterns which follow a certain sequence:

Hypotrophic or atrophic patterns followed abruptly (within 24 hours) by a estrogenic type, with 60% eosinophilic, karyopyknotic superficial cells, and the cellular pattern of normal pregnancy which we have described as "definite pregnancy pattern."

A study of several cases of hyperemesis (7) in which the sequence of appearance of cellular patterns appeared without hormonal therapy has made it possible for us to indicate an etiopathogenic hypothesis of said disease.

G. Preeclampsia and eclampsia: No alterations are found in the cytological pattern of pregnancy (2, 8). In cases of early, normal spontaneous labor, no cytological signs of placental senility were found, probably because in such toxemias the initial mechanism of labor is different from that found in physiological conditions.

H. Hydatiform mole: We studied only one case with a cytological pattern characterized by a high Karyopyknotic Index, more than 70% without navicular or oyster cells and few eosinophilic, karyopyknotic superficial cells.

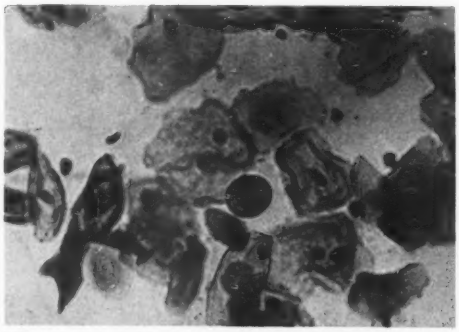

Fig. 1. Urocytogram. First week of pregnancy.

Fig. 2. Urocytogram. Fourth to eighth (or ninth) week of pregnancy.

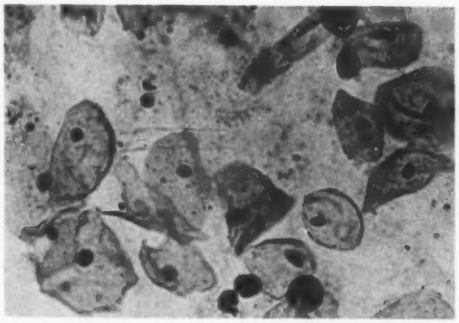

Fig. 3. Urocytogram. Tenth to twelfth week of pregnancy.

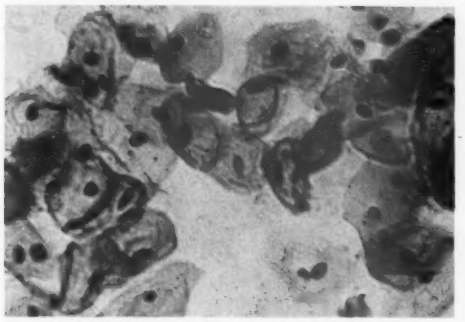

Fig. 4. Urocytogram. 14th to 38th week of pregnancy. Pregnancy pattern.

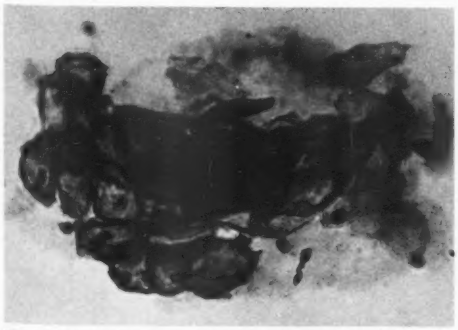

Fig. 5. Urocytogram. Typical cell cluster of a pregnancy pattern.

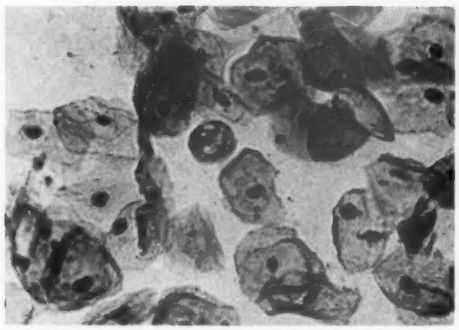

Fig. 6. Urocytogram. Pregnancy at term. Slight signs of "placental senility."

Fig. 7. Urocytogram. Pregnancy at term. Marked signs of "placental senility."

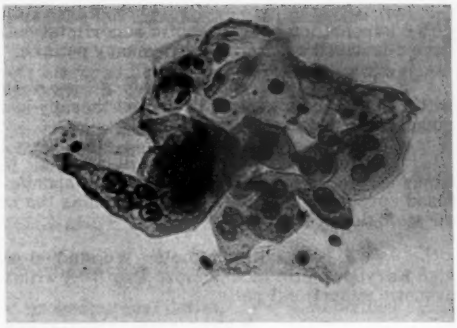

Fig. 8. Urocytogram. Labor.

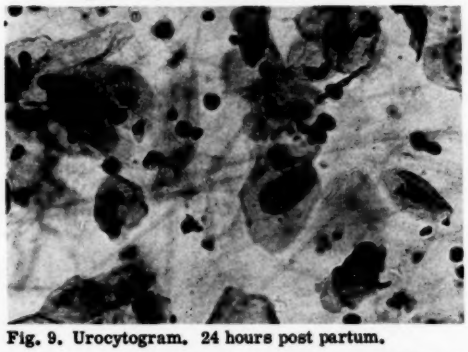

Fig. 9. Urocytogram. 24 hours post partum.

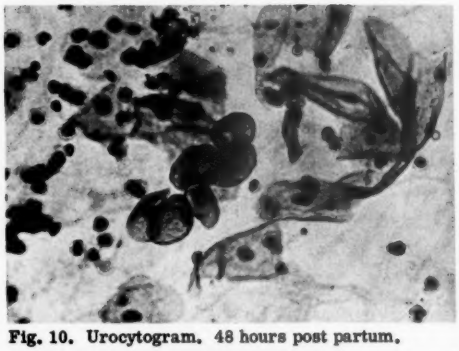

Fig. 10. Urocytogram. 48 hours post partum.

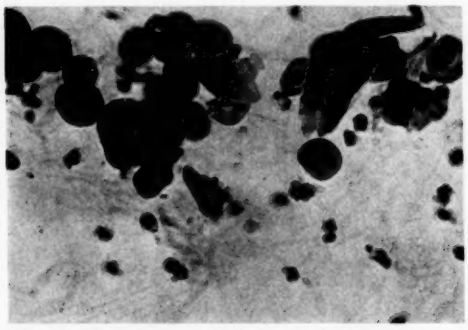

Fig. 11. Urocytogram. 72 hours post partum.

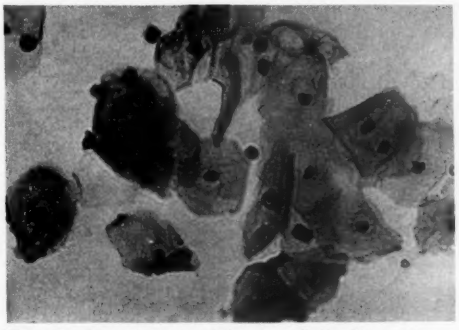

Fig. 12. Urocytogram. Threatened abortion: progesterone deficiency.

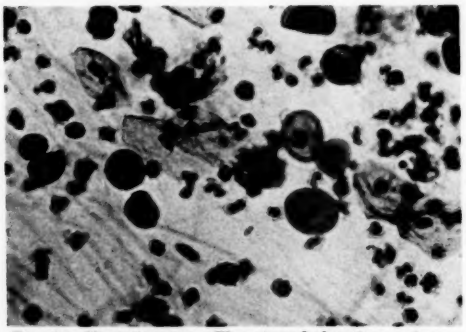

Fig. 13. Urocytogram. Threatened abortion: estrogen and progesterone deficiency.

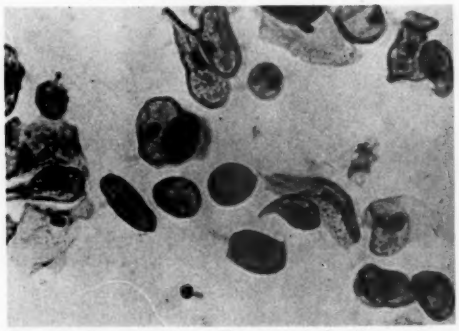

Fig. 14. Urocytogram. Threatened abortion: estrogen deficiency.

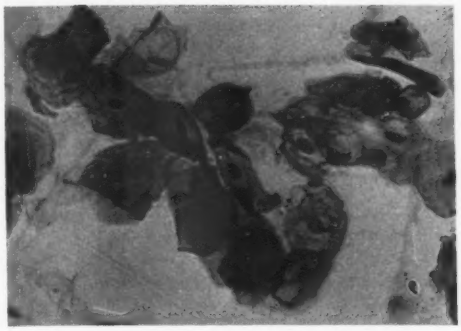

Fig. 15. Urocytogram. Threatened abortion: no hormonal imbalance.

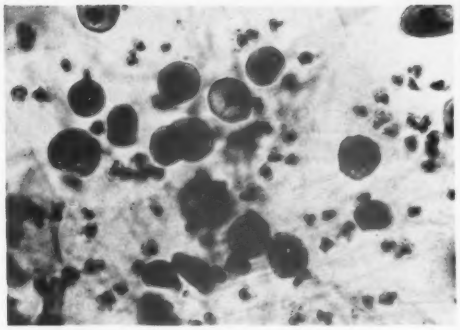

Fig. 16. Urocytogram. Missed abortion.

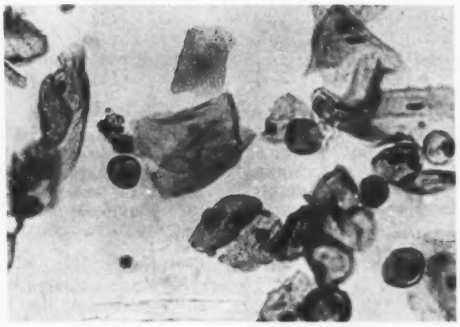

Fig. 17. Urocytogram. Threatened premature labor.

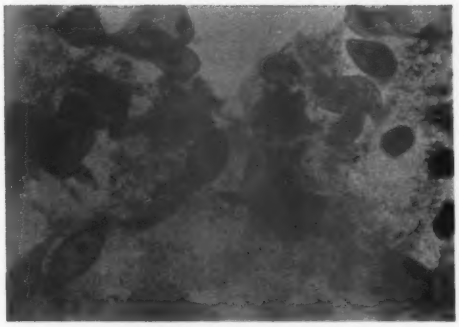

Fig. 18. Urocytogram. Hyperemesis gravidarum: hypotrophic pattern.

Fig. 19. Urocytogram. Hyperemesis gravidarum: estrogenic pattern.

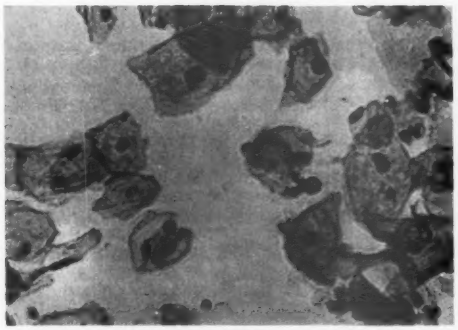

Fig. 20. Urocytogram. Hyperemesis gravidarum: pregnancy pattern.

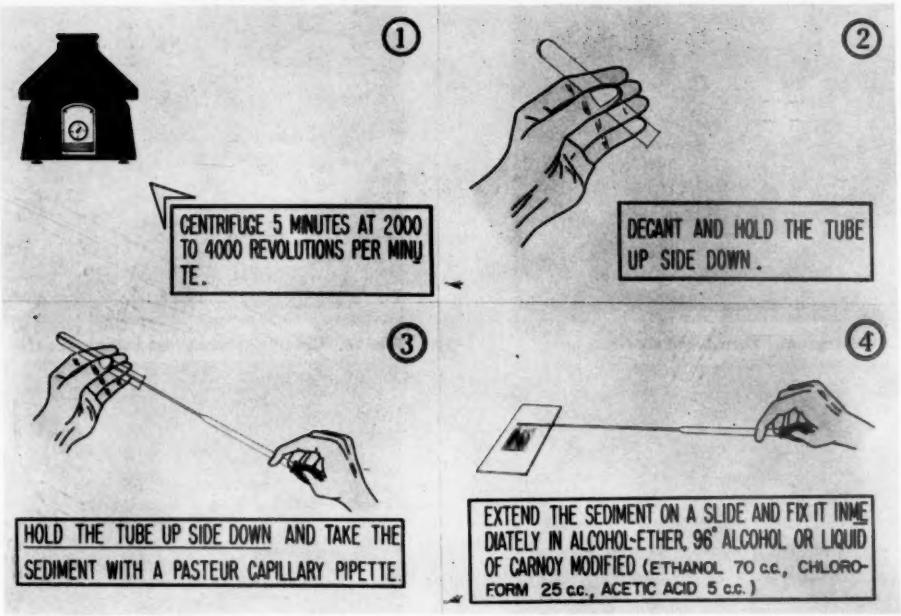

Fig. 21

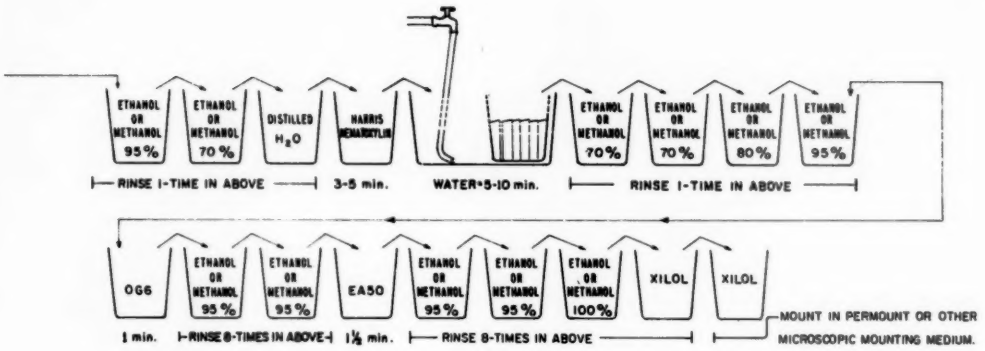

Fig. 22

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CORRELATIVE STUDIES ON VAGINAL, URETHRAL, ORAL SMEARS AND SMEARS FROM THE URINARY SEDIMENT DURING NORMAL PREGNANCY

PIERO SORA
Pavia, Italy

The present studies concern approximately one hundred women at every stage of pregnancy, or at the beginning of labor (1). During normal pregnancy the cytological picture of vaginal and urethral smears presents very similar features.

The navicular cells of pregnancy, in significant number for diagnosis, appear at the end of the first month in the urethral smears and during the second month in the vaginal smears, (in which the spindle-shaped cells of the luteal type are increasingly present before that time). These cells, found in the vaginal smears of women during the first month of amenorrhea, have great diagnostic value, in the opinion of the author. They mean that the corpus luteum has continued its hormonal function beyond the usual time and, therefore, must be considered as a sign of a relative hyperluteinism from the initial pregnancy, or of a hyperluteinism linked to the presence of active luteinic cysts in the ovaries.

The cytology of oral smears does not show any really typical changes during pregnancy, since navicular cells have never been found in oral smears. The cytological patterns of smears from the urinary sediment show evident changes at every stage of pregnancy, but they are only significant in a certain number of cases (65%). Therefore, this cannot be used as an early pregancy test.

The changes in vaginal cytology that appear near term and at the beginning of labor (increase of eosinophilic cells) are, in the opinion of the author, not signs of a relative hyperestrinism, but of lost vitality of the superficial layers of the hypertrophic vaginal epithelium, which tends to be rapidly destroyed along with those other structures associated with puerperal conditions.

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DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

Urethral smears often show a lower grade of proliferation than vaginal smears during pregnancy. This may explain why the typical changes of pregnancy in the urethral smear can be recognized earlier. Often cells, taken from urethra during pregnancy, resemble the parabasal cells more than the intermediate ones. Besides, the urethral smears can usually be visualized more clearly than the vaginal smears and can more readily be scrutinized than vaginal smears which may show Trichomonas infection or other inflammatory cell changes.

In our experience, smears from urinary sediment rarely show typical pregnancy cytology; more often than not, one sees a variable picture of cells from all layers of the epithelium, superficial as well as parabasal cells.

Oral smears do not show any changes typical of pregnancy. They show mainly large cells of the intermediate type with numerous eosinophilic cells with prepyknotic nuclei. This picture is seen, with few exceptions, during pregnancy and in the menopause, as well as in the menstruating female.

NO CLOSING REMARKS

FUTURE SYMPOSIA

The following Symposia by Correspondence are being prepared at this time or are planned for the future of ACTA CYTOLOGICA.

SYMPOSIUM BY CORRESPONDENCE ON THE EFFECTS OF ENDOGENOUS ESTROGENS ON THE VAGINAL EPITHELIUM (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON VARIOUS TECHNIQUES OF OBTAINING MATERIAL FOR CYTOLOGICAL STUDIES (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON CARCINOMA IN SITU AND SO-CALLED PRECANCEROUS LESIONS (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON ENDOCERVICAL ADENOCARCINOMA (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON TRAINING OF CYTOTECHNOLOGISTS (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON THE EFFECTS OF PREGESTATIONAL AGENTS (ENDOGENOUS AND EXOGENOUS PREGESTATIONAL SUBSTANCES) (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON THE ORGANIZATION OF A LABORATORY OF EXFOLIATIVE CYTOLOGY

SYMPOSIUM BY CORRESPONDENCE ON CYTOLOGY OF THE RESPIRATORY TRACT

SYMPOSIUM BY CORRESPONDENCE ON SEX CHROMATIN

SYMPOSIUM BY CORRESPONDENCE ON GASTROINTESTINAL CYTOLOGY

SYMPOSIUM BY CORRESPONDENCE ON COMPARATIVE DIAGNOSTIC ACCURACY, EFFICIENCY AND SPECIFICITY OF TECHNIQUES FOR EARLY CANCER DETECTION.

SYMPOSIUM BY CORRESPONDENCE ON CYTOLOGY OF THE ASCITIC FLUID

SYMPOSIUM BY CORRESPONDENCE ON HISTIOCYTES

Individuals interested in participating in the above Symposia by Correspondence are invited to contact the Editorial Office for details. The six symposia listed on the top of this list are already closed; no new participants can be accepted. However, participants for the latter seven symposia are welcome, and may be listed upon request.

There will be no more listing of details concerning the FUTURE SYMPOSIA in the journal because space does not permit listing all participants, topics, and the various deadlines for each individual symposium. This information is readily available by writing to the Editorial Office, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A. Inquiries are invited.

SYMPOSIA UNDER CONSIDERATION

The following symposia have been suggested for consideration, but are not listed in the order of preference or chronology. The readers are invited to inform the Editorial Office in which of the symposia they would be most interested, so that an order of preference may be tentatively arranged. The following topics do not include the ones which are already scheduled, and therefore listed under **FUTURE SYMPOSIA.**

Symposium on Tadpole-Shaped Squamoid Cells.
Symposium on Cytological Studies in Amenorrhea.
Symposium on Cytology of Malignant Tumors of Ovary and Tubes.
Symposium on Extra-Genital Cytology of Metastatic Gynecological Lesions.
Symposium on Phasemicroscopy and Other Special Microscopic Techniques.
Symposium on Training of Exfoliative Cytologists.
Symposium on Cytological Changes due to Microbiological Factors.
Symposium on Cytological Microphotography.
Symposium on Cytological Terminology for Hormonal Evaluation.
Symposium on Quantitive Cytochemistry of Exfoliated Cells.
Symposium on Vaginal Cytology During Childhood.
Symposium on Cytology of Exudates.

ABSTRACTS

This portion of ACTA CYTOLOGICA includes abstracts (approximately 150–300 word each) of papers, either recently published or accepted for publication. Authors are invited to submit their own abstracts. Authors are requested to forward to the Editorial Office a complete manuscript or reprint of the original paper together with their abstract. All figures should be included.

The Editorial Office maintains a *free Literature Service* for distribution of available papers to cytologists. Authors are requested to send a minimum of 10 reprints, if possible, 150 copies of published papers to the Editorial Office. The Literature Service will make photostatic reproductions of papers which are unobtainable whenever possible.

RÉSUMÉS

Cette rubrique des ACTA CYTOLOGICA contient des résumés (d'environ 150 à 300 mots) de publications qui ont été récemment publiées ou acceptées pour la publication. Les auteurs sont priés de présenter leurs résumés *en anglais*. Les auteurs sont invités à faire parvenir au bureau de rédaction, en même temps que leur résumé, un manuscrit complet comprenant toutes les illustrations ou un tiré-à-part du travail original.

Le bureau de rédaction entretient *un service gratuit d'information littéraire* pour la distribution aux cytologistes de toute publication disponible. Les auteurs sont priés d'adresser au bureau de rédaction un minimum de 10, si possible 150 copies ou tirés-à-part de travaux publiés. Le service de documentation fera dans la mesure du possible des photocopies des publications épuisées.

ZUSAMMENFASSENDE BERICHTE AUS DER ZYTOLOGISCHEN LITERATUR

Dieser Teil der ACTA CYTOLOGICA beinhaltet zusammenfassende Berichte (von etwa 150 bis 300 Worten) von wissenschaftlichen Veröffentlichungen, die entweder schon publiziert oder zur Publikation angenommen worden sind. Autoren sind hiermit eingeladen, Zusammenfassungen ihrer Arbeiten (*in englischer Sprache*) an die Schriftleitung zu senden. Die Autoren sind gebeten, der Schriftleitung das vollständige Manuskript mit allen Abbildungen oder den Sonderdruck der Arbeit einzureichen.

Die Schriftleitung unterhält einen *kostenlosen Literatur-Dienst* zur Verteilung von wissenschaftlichen Arbeiten. Autoren sind gebeten, der Schriftleitung mindestens 10, möglichst aber 150 Kopien von Sonderdrucken ihrer Arbeiten einzureichen. Der Literatur-Dienst steht auch nach Möglichkeit zur Herstellung von Lichtkopien von schwer zugänglichen Arbeiten zur Verfügung.

RESUMENES

Esta parte de ACTA CYTOLOGICA incluye resúmenes (aproximadamente de 150–300 palabras cada uno) de los trabajos, publicados recientemente, o aceptados para su publicación. Los autores deberán enviar sus resumenes *en inglés*. Se requiere a los autores para que envien a la Oficina Editorial, junto con su resumen, un manuscrito completo o separata del trabajo original. Deberán incluirse todas las figuras.

La Oficina Editorial mantiene *un Servicio de Literatura, gratuito*, para la distribución de trabajos disponibles. Se ruega a los autores que envien a la Oficina Editorial un minimo de 10 copias de sus trabajos publicados y, de ser posible, 150 copias. El Servicio de Literatura hará, siempre que ello sea posible, reproducciones fotostáticas de los trabajos que los autores no puedan obtener.

CANCER CYTOLOGY

THE HISTOMORPHOLOGICAL BASIS FOR THE SUSPICIOUS CYTOLOGICAL FINDINGS (UEBER HISTOMORPHOLOGISCHE GRUNDLAGEN DES ZYTOLOGISCHEN VERDACHTS-BEFUNDES)

F. BAJARDI, Geburtsh. u. Frauenhlkde. 19:669, 1959

1. In a series of patients examined by the author a malignant tumor of the internal reproductive tract or at least an atypical epithelium in the region of the uterine cervix was confirmed histologically in 50.6% of the cases in which cytological examinations (Papanicolaou Class III) had aroused suspicion.

2. In a further group of 26.3% of the cases it was possible to demonstrate histologically the source of the suspicious cytological patterns in the form of hyperactive squamous epithelium or undifferentiated regenerative epithelium. In only 23.1% of the cases did the cause of the suspicion aroused by cytological examinations remain obscure.

3. The author draws attention to the frequently observed close relationship between hyperactive ("restless") and carcinomatous epithelium and to the probable significance of the hyperactive ("restless") epithelium and undifferentiated regenerative epithelium. The importance of detecting and following up these types of epithelia is stressed. (Author's abstract.)

CYTOLOGICAL DIAGNOSIS OF PROSTATIC CARCINOMA

J. BAMFORTH - Annals of the Royal College of Surgeons of England 23:248, 1958

Various cytological types of malignant cells, found by microscopical examination of smears of prostatic secretion obtained by massage of the organ in patients complaining of prostatic symptoms, have been described and pictured. All types were verified by histological examination of tissue removed for biopsy, or by prostatectomy or at autopsy.

In the majority of cases the typical "crowded cast" of malignant cells, as featured by previous workers, has been found but on the whole the appearance of the malignant cells seen in smears from different cases were subject to considerable variation.

The effect of endocrine treatment on the prostatic smear has been studied in a few cases and the subsequent degenerative changes in the malignant cells described and illustrated.

Smears from 513 patients were obtained but of these 177 were discarded as being inadequate for cytological examination. This constitutes a major problem. The technique of prostatic massage is difficult to acquire and varies in different hands. Of the remaining 336 cases, 121 were reported as positive and 39 as suspicious; of these, 62 and 8 respectively have been confirmed to date by histology or by the appearance of x-ray metastases. There is great difficulty in the follow-up of these patients and it is possible that some of the positive results may represent cases of latent carcinoma. (Author's abstract.)

A NEW AND RAPID METHOD FOR DIAGNOSIS OF VAGINAL AND CERVICAL CANCER BY FLUORESCENCE MICROSCOPY

LUDWIG VON BERTALANFFY, MARIANNA MASIN and FRANCIS MASIN - Cancer 11:873, 1958

Description of the acridine-orange (AO) fluorescence method of cytological cancer detection developed by the authors.

Technique: Fluorescence microscope with mercury maximum pressure burner, Filter B G 12 and suppression filter. Smears are:

1. Fixed in ether-alcohol.
2. Passed through graded alcohol 80, 70 and 50% and distilled water.
3. Rinsed briefly in 1% acetic acid, then washed in distilled water.
4. Stained for 3 minutes in 0.01% acridine-orange in phosphate buffer.
5. Transferred for 1 minute into M/15 phosphate buffer (pH=6) to remove excessive dye.
6. Differentiated twice with M/10 $CaCl_2$ for thirty seconds (or longer) and washed with buffer.
7. Washed with phosphate buffer and mounted under a cover glass in a drop of buffer.
8. Examined under the fluorescence microscope.

The main cell types of normal epithelium, atypical epithelium, and squamous cell carcinomas, as demonstrated in smears by the AO method, are described.

The advantages of the AO method are discussed. (Authors' abstract.)

A RAPID FLUORESCENCE-MICROSCOPIC METHOD FOR DIAGNOSIS OF GYNECOLOGICAL CARCINOMA. (EINE FLUORESZENZMIKROSKOPISCHE SCHNELLMETHODE ZUR DIAGNOSE DES GYNAEKOLOGISCHEN CARCINOMS)

LUDWIG VON BERTALANFFY, - Klinische Wochenschrift 37:469, 1959

The paper reviews the acridine-orange (AO) fluorescence method introduced by the author, and its clinical use. Some of the advantages of this method are: Essential reduction of the time for processing (6 minutes per individual slide) and screening (3 minutes average); a highly polychrome picture clearly showing morphological criteria and obtained by application of one dye only in a simple and rapid procedure; introduction of cytochemical characteristics (RNA content) as a new diagnostic criterion; red blood cells do not interfere with reading the slide; easy distinction of normal and suspicious smears under low scanning powers; rapid cytological diagnosis in the doctor's office; reduction of training time of screening personnel; etc.

The diagnostic reliability of the method was tested by double-blind comparison of AO and Papanicolaou diagnoses of identical smears, both in materials selected with emphasis on suspicious and malignant smears (598 cases) and in a supposedly well population (1750 cases). Differences between readings obtained by the two methods were small (cytologically negative 94.4% AO vs. 95.5% Pap.; positive 1.3% AO vs. 1.2% Pap.; doubtful 3.3% AO vs. 2.3% Pap. in the last mentioned material). Increase in Class III probably indicates earlier detection of unsuspected malignancies by the fluorescence method. Comparison with biopsy (58 cases) further confirmed the accuracy of the AO method.

Addition made in present abstract. Reports from other laboratories, e.g. from the Walter Reed Army Hospital on 5,491 smears leading to adoption of the AO fluorescence method for all routine exfoliative cytology at this hospital (SCOPE, May 6, 1959)--have further established the accuracy of the AO method. It has been shown, in approximately 10,000 cases investigated in the author's and other laboratories that the reliability of the AO method equals that of conventional cytodiagnostic methods. (Author's abstract.)

A FLUORESCENCE-MICROSCOPIC METHOD OF CANCER DETECTION IN BRONCHOGENIC CANCERS.

LUDWIG VON BERTALANFFY and FELIX D. BERTALANFFY - Paper presented at the Second Workshop Conference on Lung Cancer Research of the American Cancer Society, Arden House, Harriman, New York, 1959

In application to exfoliated material of the respiratory system (sputums, bronchial secretions, etc.) the acridine-orange (AO) fluorescence method was found to present the same advantages as described in application to gynecological cancer. Malignant cells are characterized by their intense cytoplasmic fluorescence which is flaming orange to red in most types of bronchogenic cancer. The AO method appears to

facilitate differential diagnosis of cells such as basal undifferentiated cells and macrophages (histiocytes) which are apt to present difficulties with conventional cytodiagnostic methods.

Main cell types in sputums, bronchial secretions, etc.

as observed with the AO fluorescence method:

Ciliated columnar cells	Cytoplasm:	reddish brown, often reticulated.
	Nuclei:	green
	Cilia:	brown
Basal cells of epithelium of respiratory system	Cytoplasm:	Faint reddish brown
	Nuclei:	yellow
Pulmonary macrophages (histiocytes)	Cytoplasm:	reddish brown (often inclusions)
	Nuclei:	from dark green (if pyknotic) to light green or yellow.
Stratified squamous epithelial cells from esophagus, pharynx and oral mucosa (in sputums)	From superficial layers:	
	Cytoplasm:	faint green
	Nuclei:	green (pyknotic)
	From basal layers:	
	Cytoplasm:	yellowish-brown
	Nuclei:	green-yellow with distinct chromatin granule
Epidermoid bronchogenic carcinoma	Cytoplasm:	bright orange-red
	Nuclei:	yellow
Adenocarcinoma	Cytoplasm:	carmine red (mucoproteins)
	Nuclei:	yellow
Anaplastic cells	Cytoplasm:	bright orange
	Nuclei:	yellow

(Authors' abstract.)

THE PROBLEM OF THE PRE-INVASIVE AND INVASIVE CARCINOMA COLLI UTERI AND THE SO-CALLED PRECANCEROUS STATES IN THE LIGHT OF HISTOCHEMICAL RESEARCH (DAS PROBLEM DES ARAEINVASIVEN UND INVASIVEN KOLLUMKARZINOM UND DER SOG PRAEKANZEROSEN ZUSTANDE IM LICHTE DER HISTOCHEMISCHEN UNTERSUCHUNGEN.)

ANTONI BIELECKI - Zentralblatt für Gynakologie 19:743, 1958

The quantity of desoxyribonucleic acid in the flat epithelium (stained by the Feulgen technique) is, in the case of pre-invasive carcinoma colli uteri, as high as in the invasive forms. There is, consequently, not a significant difference in the dynamics of the cellular division in either of the malignant processes. Our experience concerning the dynamics of the cellular division is in complete accord with the research results of other authors, who also have not found any difference between these two forms of malignant spread with regard to the proliferation of tissue, as well as to the metabolism of tissue and cells. According to our experience, the established proportion of DNA in the cellular chromosomes (stained by Feulgen technique) may be applied as a guiding test in the clinical and therapeutical evaluation in the so-called precancer states, and in cases of paratypical changes affecting the epithelium of the portio. (Author's abstract.)

THE ATYPICAL TRANSFORMATION ZONE (UEBER DIE ATYPISCHE UMWANDLUNGSZONE)

E. BURGHARDT, Geburtsh. u. Frauenhlkde. 19:676, 1959

Increasing experience of colposcopic diagnosis has shown that atypical epithelia are to be found not only together with the patterns of the so-called matrix regions, but also inside transformation zones. This has made it necessary to work out new diagnostic factors on the notion of the "atypical transformation zone." The knowledge of the histological substrate of typical zones which yielded cytological "positive" findings, have revealed details that became important for colposcopic diagnosis. Statistics relating to cases diagnosed at an early stage in 1956 reflect the results which were achieved by an intensive study of this problem. (Author's abstract.)

DYSKAROSIS IN CONNECTION WITH DYSPLASIA AND PREINVASIVE CARCINOMA (DIE "DYSKARYOSE" IM ZUSAMMENHANG MIT "DYSPLASIE" UND PRAINVASIVEM CARCINOM)

B. COUTIFARIS and L. COUTIFARIS - Zenttralblatt für Gynakologie, Date of Acceptance for publication, December, 1958

Thirteen thousand, one hundred and eleven women have been examined by vaginal and cervical smears. Fifty-eight cases belonged to Class III.

In Class III we classify the cases that showed "dyskaryosis" of the parabasal cells. In these cases our reports were as follows: "Dyskaryosis, CLASS III." Twelve women out of the 58 had the above report. Histologically all of them had intraepithelial carcinoma.

The report given the twelve cases was: suspicious cells of a malignant neoplasm were found. CLASS III. Histologically all of them had carcinoma (two of the corpus, eight of the cervix, one of the vagina, and one of the ovaries).

In 14 cases our report was: dyskaryotic changes of the superficial and navicular cells of the squamous epithelium. ATTENTION, CLASS III. Of these cases, eight have been proven histologically to have a dysplasia of the cervix, four cervicitis and two were pregnant.

In 20 of our cases, biopsies were not performed because the patients never answered our letters.

The importance of "dyskaryosis" of the parabasal cells is pointed out. (Authors' abstract.)

CERVICAL CARCINOMA: CANCER CELLS IN THE CIRCULATING BLOOD

A. W. DIDDLE, D. M. SHOLES, J. HOLLINGSWORTH and S. KINLAW, Am. J. Obst. & Gyn. 78:582, 1959

Tumor cells were isolated from the circulating blood from 9 out of 14 untreated patients with invasive, endophytic, epidermoid carcinoma. These cells were found only in the venous blood from the drainage site of the tumor. None was found in the external iliac, femoral, or antecubital veins. Cells were not found in a patient with an in situ lesion or those without a malignancy. Occasionally atypical cells were found in patients with invasive lesions that appeared relatively early clinically. These same growths proved to be more advanced by adequate histologic and gross pathologic study. It is wondered if circulating tumor cells in women with invasive cancer have prognostic value. (Authors' abstract.)

CYTOLOGY: AN EVALUATION AND FOLLOW-UP OF 25,000 GENITAL SMEARS

WILLIAM J. ESTRADA, S. J. SKINNER, GEORGE V. MILLER, and A. M. FARIS - Am. J. Obst. & Gyn. - 77:175, 1959

A study of 25,000 consecutive genital smears taken in the Houston area, by 82 physicians, in the private practice of medicine is presented. The smears on 17,000 women were submitted to a single group of pathologists.

The study revealed that 262 of the total smears were positive or suspicious, and of this group 214 were associated with premalignant or malignant lesions of the genital tract. Of this group 65% were taken from cervices with unsuspected lesions.

Proper follow-up of positive and suspicious smears is stressed in that 19% were improperly followed-up. The authors feel that the best method of handling positive or suspicious smears is to do an endometrial and cervical biopsy on an outpatient basis; if this is negative for invasive carcinoma, then a fractional curettage of the uterus and cold knife conization of the cervix is done in the hospital.

There were 32 positive smears in the below 30 year age group, and routine smears were stressed for this group as well as the above 30 year age group. The value of repeating smears at regular intervals in patients with previously negative smears was shown, in that 17 such patients developed a positive smear. (Authors' abstract.)

FATE OF WOMEN WITH POSITIVE CERVICAL CYTOLOGY

JAMES HENRY FERGUSON and HARVEY LOZMAN - South Med. Assoc. 51:296, 1958

An investigation among 5,473 cervical cytologic examinations revealed 169 positive smears; 85 with a completed follow-up. Of these, 76 are alive and 9 are dead. Of all positive cases, 1/3 were under age 30, 33 had invasive squamous cell carcinoma of the cervix. There was one carcinoma of the cervix to

166 examinations or 6.1/1000.

The chief diagnoses in the 85 women with completed follow-up were invasive carcinoma in 25, cervicitis in 21, intraepithelial carcinoma in 9.

Forty-three of the 85 women (51%) had false-positive cytology reports; no cancer appearing at the time of completed follow-up. They are being followed by the Positive Cytology Registry.

Reversal of positive cytology to negative occurred in 39 women during an average follow-up of 32 months. In 20 no tissue diagnosis was ever made. There was one instance of intraepithelial carcinoma, 2 invasive carcinoma, 15 had cervicitis, and one a cervical polyp. Twenty-three had no treatment.

Of the 85 women with completed follow-up, 12 had had 17 pregnancies since the first positive smear. These patients include one with invasive carcinoma, two with intraepithelial carcinoma. All 12 are alive and well.

Since eight positive smears were found in the first postpartum visit, and the suspicion that more abnormal smears are obtained from pregnant than non-pregnant women of comparable age, the antepartum and postpartum clinics are a rich source of positive smears. The authors feel that all positive smears should be followed indefinitely. (Authors' abstract.)

A CYTO-PATHOLOGIC STUDY OF TOBACCO TAR-INDUCED LESIONS OF THE UTERINE CERVIX OF MOUSE

I. KOPROWSKA and J. BOGACZ, - J. Natl. Cancer Inst. 23:1, 1959

Neoplastic lesions were induced in cervical and vaginal mucosa of C3H mice by applying intravaginally crude tobacco tar five times weekly for forty-four weeks under strict visual control through an infant size otic speculum. Weekly vaginal smears of tobacco tar-treated mice, benzpyrene-treated mice and untreated mice of the same strain were scored for presence of eleven cytologic criteria of malignancy during the period of the carcinogenic treatment. Histologic evaluation of induced lesions was based upon three different sets of standards as follows:

1. Lesions were compared to benzpyrene-induced early cervical carcinoma developed after specified gradually-increasing number of weeks of benzpyrene treatment.

2. They were classified by Suntzeff's grades according to the extent of epithelial downgrowth.

3. Diagnoses were made according to von Haam's classification for benzpyrene-induced cervical carcinoma.

It was shown that cytologic findings and histologic lesions induced by tobacco tar in the cervix and/or vagina of C3H mice were strikingly similar to those observed during the development of 3-4 benzpyrene-induced cervical carcinoma. Since tobacco tar-induced lesions develop after prolonged treatment and persist for several months, their use may offer a better opportunity for morphologic and biologic studies of carcinoma in situ than the use of rapidly-growing neoplastic lesions induced by strong carcinogens such as 3-4 benzpyrene or methylcholanthrene. Further study is necessary to determine the final stages of development of neoplastic lesions induced by tobacco tar.

It was demonstrated that the uterine cervix is a suitable site for the study of carcinogenic properties of tobacco tar. (Authors' abstract.)

CLINICAL AND PATHOLOGIC SIGNIFICANCE OF ANAPLASIA (ATYPICAL HYPERPLASIA) OF THE CERVIX UTERI

D. G. McKAY, B. TERJANIAN, D. POSHYACHINDA, P. A. YOUNGE and A. T. HERTIG - Obst. and Gyn. 13:2, 1959

Between the years 1945 and 1954 there were 243 cases of anaplasia of the cervix at the Free Hospital for Women. This is an occurrence rate of 1.2% of all cervices examined pathologically. The average age was 34.9 years, which is three years younger than the average age of patients with carcinoma in situ and 13 years younger than that of patients with invasive carcinoma of the cervix. The clinical outcome was traced in 129 of these patients on whom adequate follow-up data was available. In 20.2% of the cases, anaplasia disappeared; i.e., it was not found in the hysterectomy specimen or in smears and biopsies taken in 1957. In 3.8% anaplasia progressed to carcinoma in situ (4 cases) or invasive carcinoma (1 case). In 32.5% anaplasia was associated with a coexistent carcinoma in situ (37 cases) or invasive carcinoma (5 cases). Seventeen per cent of these were found incidentally in the hysterectomy specimen which was removed for reasons other than neoplasm of the cervix. Anaplasia persisted in 26.4% of the cases; i.e., it was found in the hysterectomy specimen or cone specimen on an average of six months after the initial biopsy. Exfoliative cytologic examination of 67 patients with anaplasia alone was positive at some time during the period of study in 52.3%, suspicious in 14.9%, and negative in 32.8%. When four or more

smears were taken over a period of 19 months or more, the smears were 85 to 100% accurate in detecting this lesion. Ten were primarily diagnosed by exfoliative cytology, 19 were simultaneously discovered by smear and biopsy, and 38 were initially detected by biopsy. Cervical aspirations were slightly more sensitive in detecting anaplasia than vaginal smears. (Authors' abstract.)

COMPARATIVE STUDIES OF VAGINAL SECRETIONS IN SMEARS AND IN PARAFFIN SECTIONS (VERGLEICHENDE UNTERSUCHUNGEN VON VAGINALSEKRET IN SCHNITT - UND AUSSTRICH-PRÄPARATEN)

HEINRICH WALTER MIEBACH - Thesis - Med. Akad. Düsseldorf, Germany, 1958

Comparative investigations were made of smears and paraffin sections of both normal vaginal secretions and those containing carcinoma cells. The smears were fixed in ether-alcohol and stained with a modified Papanicolaou technique. The sections, prepared from smeared material fixed in ether-alcohol and imbedded in paraffin, were stained the same as the smears. As shown in seven microphotographs, cellular differentiation can be made as readily in the sections, from the same morphological characteristics which are diagnostic in smear preparations. In the paraffin sections, cell groups were very often encountered. These could not be interpreted in the smears because of their thickness. The cells composing these groups were well preserved and suggested, that they were fixed in the living state. It is emphasized, that fresh cells are essential for investigative purposes; they serve as a basis for proposed investigations with the electron microscope. Disadvantages of the paraffin sections are the cellular shrinkage and the difficult technique. (Author's abstract.)

A SOURCE OF FALSE POSITIVES IN CYTOLOGIC INTERPRETATION

M. MILIGAN, L. A. CARROW and V. EGGERS, Am. J. Obst. & Gyn. 78:599, 1959

1. In our laboratory in the past, the so-called "blue blobs" were known to have been responsible, in four cases, for a cytologic diagnosis of carcinoma and in four other cases for the diagnosis of moderately atypical squamous cells. In those cases where tissue study was available, the biopsy and/or surgical specimens were negative.

2. "Blue blobs" are found more frequently in postmenopausal women; this was true in 94.3% of the cases in this study.

3. Trichomonas vaginalis were recognized cytologically in 86.8% of our series of 53 patients whose smears contained "blue blobs."

4. Estrogenic stimulation appears to decrease the number of "blue blobs", or to eliminate them entirely. Therefore, the administration of estrogens might be utilized in making a differential diagnosis in the cases where the report was based on atypical free nuclei. (Authors' abstract.)

CONCERNING THE PARANUCLEAR HALOS SEEN IN PRECANCEROUS LESIONS OF THE COLLUM UTERI (KOLLUM'UN PREKANSEROZ LEZYONLARINDA RASTLANILAN PARANUKLEAR HALOLAR HAKKINDA)

NURI SAĞIROĞLU - Tip Fakultesi Mecmuasi 21:904, 1958

There are two types of halos seen in the cells. In one type a small area is covered around the nucleus, usually in Trichomonas infestation of the vagina and placed spherically around the nucleus; this is probably the true perinuclear halo.

The second type, with irregular and sharply indented borders, also covering a large area, was discussed in a previous article, as a membranal and cytoplasmic cellular defect, induced generally, by mechanical factors under suitable pathological conditions of the uterine cervix. The term "Paranuclear Halo" has been introduced to differentiate between this and the first-named haloed cells.

Paranuclear halos are often observed in precancer cells. Thirty percent of precancer cases studied exhibited paranuclear halos. Some investigators believe the paranuclear halo may be the first cell-sign of cancer development.

In three of our research patients, spontaneous regression of precancer cells with paranuclear halos occurred. Only normal or simple inflammatory cells are now seen in the smears. Regression was accomplished in 5-7 months.

The author postulates that paranuclear halos may not be the earliest cell-signs of cancer development in cases as above described. On the contrary, they probably are seen during the recovery stage of a precancerous lesion, or chronic inflammation. (Author's abstract.)

THE NATURE OF THE 'PERINUCLEAR HALO' FURTHER CLINICAL, CYTOLOGICAL AND PATHOLOGICAL STUDIES

NURI SAĞIROĞLU - Am. J. Obst. & Gyn. 77:159, 1959

One hundred and nine research patients were clinically and cytologically studied by the cervical-scraping-smears method. The cells from 33 (30%) of the cases displayed halos which covered large areas and showed sharp borders.

Ayre has accepted this type of halo as important evidence corroborating his precancer cell complex.

Papanicolaou has called them "Perinuclear Cavitations."

Koss and Durfee have established a special category for cases showing cells with perinuclear halos; they have labelled these cells koilocytotic atypia and they believe this type of halo is the result of altered cellular metabolism.

With regard to my studies, the perinuclear halos under discussion are the result of membraneous, cytoplasmic and rarely, nuclear defects. The halo borders, as seen in photomicrographs appearing with the original manuscript, are large, irregular and frequently, indented deeply as though the membrane had been roughly torn. Some cells have no nucleus within the halo. Folded cells with halos do not show membrane covers on their nuclei.

The suitable condition of the uterine cervix, as chronic nonspecific inflammation in which some anaplastic dead and macerated cells may be found on the cervical surface, plus some scraping factors, probably are responsible for cellular defects or the production of halos. (Author's abstract.)

DETECTION OF GYNECOLOGIC CANCER BY FLUORESCENCE MICROSCOPY

WALTER SUSSMAN - Am. J. Obst. & Gyn. 13:273, 1959

Fluorescence microscopy using the ultraviolet light lamp and the organic dye, acridine-orange, provides a simple and rapid mass screening technique for the earlier detection of cancer. Acridine-orange (AO) has a special affinity for the two nucleic acids of the living cell, Desoxyribonucleic (DNA) found in the nucleus, and ribonucleic (RNA) found in the cytoplasm and nucleolus. The AO gives RNA an orange-red fluorescence in the cytoplasm and nucleolus, and DNA a green fluorescence in the chromatin. The brilliance facilitates scanning. Normals can be by-passed quickly.

The total staining technique, using alcohol 80%, 70% and 50% distilled water, acridine-orange solution 0.1% and phosphate buffer, takes eleven minutes. The research work in progress, in gynecological office practice, was done in the doctor's office and reports were available in 45 minutes from the time of taking the smear. In 1421 cases there were 1274 normals, 137 atypicals (recalled every three months), 7 suspicious (biopsy and follow-up) and 3 malignant. It is applicable for all wet and dry smears, and frozen and paraffin sections, regardless of origin. Because of the rapidity of staining and scanning, the technique should prove useful for mass screening. (Author's abstract.)

HORMONAL CYTOLOGY

DIAGNOSTIC VALUE OF CYTOHORMONAL PICTURES IN THE CASES OF EXTRAUTERINE PREGNANCY (WARTOSC ROZPOZNAWCZA OBRAZOW CYTOHORMONALNYCH W PRZYPADKACH CIAZY POZAMACICZNEJ)

ANTONI BIELECKI, Ginekologia Polska, 30:161, 1959

In the material from 37 cases of supposed extrauterine pregnancies, the cytohormonal evaluation of vaginal smears in 32 cases corresponded to Friedman's reaction and to the clinical course. In three cases the result obtained was falsely positive and in two cases falsely doubtful. Of the general number of 37 cases, extrauterine pregnancy was diagnosed in seven patients. With regard to the interpretation of cytohormonal pictures, no characteristic features could be established in the cases of extrauterine pregnancy. The diagnosis is made possible by the presence of numerous cells from the intermediary layer - navicular cells - "pregnancy cells."

The author emphasizes that the value of the method as an auxiliary diagnostic test with this restriction is less binding when compared with the biological reaction. (Author's abstract.)

HORMONAL EVALUATION OF A MALE PATIENT BY MEANS OF CYTOLOGY (HORMONELLE ZYTODIAGNOSTIK BEIM MANN)

K. KLEIN, Wiener Klinische Wochenschrift, 71:425, 1959

Cytologic and cytometric examinations of 600 smears of the oral mucosa in cases of male patients between 18 and 80 years of age were made before and after application of hormones. The cell material taken from the cheek mucosa was stained by the method of Shorr.

We came to the conclusion that the male buccal mucosa reacts to estrogenic and androgenic stimulation on principle in a similar manner to the female oral or vaginal epithelium.

Therefore it is possible to establish the following facts with cytological methods:

1. Disturbances of the male hormone balance (for example, hyperestrogenemia in cases of cirrhosis of the liver, male climacteric stage).

2. The effect of the administration of estrogenic and androgenic substances.

But as the sensitivity of the oral mucosa of the male patient is less intensive than that of the female one, it is necessary to supply high dosages of hormones (substances with prolonged activity), in order to realize a recordable cytologic effect.

Summing up we can say, that by means of the oral cytology we find an adequate morphologic way to determine the qualitative hormonal state in male patients in each individual case and to give a guide to therapy with sex hormones.

Further tests are actually in progress to study the cytologic changes in the male buccal mucosa caused by hormones of the adrenal gland, progestative substances and ACTH. (Author's abstract.)

CYTOLOGY IN INFLAMMATORY REACTIONS

PREMENARCHAL VAGINITIS

WARREN R. LANG, Obst. & Gyn. 13:723, 1958

A clinical appraisal of 110 cases of premenarchal vaginitis is reported. After taking the history, each child was investigated gynecologically as follows: (1) inspection of the vulva (2) visualization of the vagina and cervix (with a Kelly cystoscope) and (3) bimanual (rectal) examination. Smears and cultures were also taken at this time. Anesthesia was necessary on only three occasions. There were no untoward effects noted from the examination. In fifteen instances the discharge was considered to be "physiologic" (leukorrheas of the newborn and of puberty). Most cases of vaginitis were of the so-called "mixed" variety with various fecal organisms found on culture. Two children had foreign body vaginitis. Treatment utilized may be categorized as: nonspecific local measures (e.g., cleanliness, witch hazel compresses, cortisone ointment) specific systemic measures (e.g., appropriate antibacterials, antibiotics) and specific local measures (e.g., carefully administered suppositories, creams, jellies, douches). Specific local methods of therapy were naturally avoided whenever possible. (Author's abstract.)

VALUE OF CYTOLOGICAL DIAGNOSIS – A GENERAL REPORT (VALEURS ET INDICATIONS DES METHODES CYTOLOGIQUES)

J. DELARUE, ORCEL and COLETTE MARSAN – Semaine Des Hopitaux

This paper is concerned with a general review of the actual value of cytological methods and reports the most important statistics on the subject.

Several tables show that the results appear considerably different according to the site of the tumor. There are numerous causes of errors, partly depending on the fact that no definite cytological criterion specific for malignancy has yet been established.

The method is mostly employed for diagnostic purposes, especially when the female reproductive tract is involved and, in these cases, the results are excellent. But technical and psychological problems are still existant in Europe and represent the principal causes of survey in the generalization of the method.

Cytology is also employed for the diagnosis of deeper cancers (gastric, eosophageal, bronchial) when no other means of early diagnosis is satisfactory.

Finally, cytological techniques are used to follow the behavior of treated cancers and in some cases, to appreciate their prognosis. This seems to be actually one of their most interesting applications. (Authors' abstract.)

CYTOLOGICAL TECHNIQUES

CYTODIAGNESIS WITH TOLUIDIN BLUE VITAL STAINING (CITODIAGNOSTICO EM MATERIAL A FRESCO CORADO PELO AZUL DE TOLUIDINA)

CLARICE AMARAL FERREIRA and ILKA MENEZES - Anais Brasileiros de Ginecologia 47:23, 1959

The authors refer to their first experience with the toluidin blue staining method of fresh cervical material. They use it in the first screening in the detection of genital cancer in external consultation at the Institute of Gynecology, University of Brazil. The toluidin blue stains almost only the nuclear chromatin, permitting a diagnosis of atypias.

This group includes 540 cases, of which 169 also were examined by routinely fixed smears. No false negatives were found. Thirteen positive cases were found with only one of them being false positive (a case of endometrial tuberculosis).

It seems to the authors that the toluidin blue vital staining method, used by Guzman in Chili, for first screening in cancer detection, is very helpful. (Authors' abstract.)

CYTOLOGICAL FINDINGS ON OSMIUM FIXED VAGINAL EPITHELIAL CELLS (CYTOLOGISHE BEFUNDE AM VAGINALEPITHEL BEI FIXIERUNG MIT OSMIUM)

HANNS JÜRGEN HANSCHKE - Geburtshilfe und Frauenheilkunde, Date of acceptance of publication, January, 1959

The cytologic findings in smears and paraffin sections made from normal vaginal mucosa and from cervical carcinoma are compared after fixation in osmium tetroxide, without staining. The sections are from paraffin imbedded material. The technique of their preparation is explained. As is shown in seven microphotographs, by use of both cytologic methods, the various types of normal and malignant cells can be differentiated equally well. Tumor cells in the smears are often altered by regressive changes. In tissue fragments they are better preserved. In the sectioned material the tissue structure is clearly evident and quite adequate for diagnostic purposes. The cellular shrinkage induced by paraffin imbedding is insignificant. The smears are best used for special morphologic problems. Since electron microscopic investigations rely essentially on osmium fixation, our studies can serve as a basis for future electron microscopic studies. (Author's abstract.)

FLUORESCENCE COLPOSCOPY AND FLUORESCENCE COLPOPHOTOGRAPHY (DIE FLUORESZENZKOLPOSKOPIE UND FLUORESZENZKOLPOFOTOGRAFIE)

R. HOHLBEIN, Geburtsh. u. Frauenhlkde. 19:656, 1959

After reviewing various aides to extended colposcopy, the author discusses fluorescence colposcopy in detail. Fluorochromization of the uterine cervix with the fluorochrome acridine orange makes it possible to differentiate between normal and atypical epithelium, as well as, within certain limits, between atypical and carcinomatous epithelium. The various findings are described. Having first surveyed the development of colpophotography, the author goes on to describe a method of documenting the findings of fluorescence colposcopy. Fluorescence colpophotograms are shown. (Author's abstract.)

A FILTER MEMBRANE TECHNIQUE FOR CYTOLOGICAL STUDY OF EXFOLIATED CELLS IN BODY FLUIDS

KERRISON JUNIPER, JR. and CLARENCE L. CHESTER - Cancer 12:278, 1959

A filter membrane technique for concentrating exfoliated cells from pleural and ascitic fluids is presented. The Millipore Filter with a 5 micron pore size was used. From 2 to 5 ml. of body fluid was sufficient to load the filter, using a vacuum no greater than 25 mm. of mercury. The cell-coated filter was fixed in a 4% solution of buffered formaldehyde solution containing 1% acetic acid. The fixed cell-coated filter was stained with a Shorr stain, dehydrated in ascending ethyl alcohols and cleared in xylene. The cleared filters were mounted on microscope slides with Permount. The cytologic detail obtained with the filter technique was unusually good, comparing favorably with that obtained by smear methods with Papanicolaou's procedure. The filter method probably will prove most useful in the cytologic study of very small quantities of body fluids because of the filters absolute concentrating and retentive characteristics. It also should be useful in extracting cells from saline washings of serous cavities. (Authors' abstract.)

A SIMPLIFIED FILTER TECHNIQUE FOR CYTOLOGIC DETECTION OF URINARY MALIGNANCIES

JOHN W. MERRITT, VAYNE B. HENDERSON and THOMAS A. SLATE, Journal of Urology, Date of acceptance for publication, January, 1959

This study was undertaken in an attempt to develop a simple, but accurate, technique for the detection of urinary malignancy. This technique eliminates previous objections regarding the value of cytology in this field of study. The procedure involves the use of the Millipore monitor and filter which allows filtration of the specimen in the private doctor's office and eliminates (1) the need for transportation of bulk samples of urine, (2) the necessity of centrifugation, and (3) allows a much larger number of epithelial elements to be collected for study. The cells remain intact permitting a high degree of accuracy in evaluation.

This preliminary study includes one hundred smears obtained from ninety-six patients submitted by a group of urologists. There were eleven Class IV or V smears obtained from seven carcinomas of the bladder and one carcinoma of the right kidney. Six Class III smears aided in the detection of six various benign atypias. Only nine smears were considered unsatisfactory.

The results to date suggest that this technique has great potentiality and should aid in making more practical the use of cytology for the detection of cancer in the urinary tract. (Authors' abstract.)

HISTOCHEMISTRY OF CELLS IN THE SPUTUM. I. QUALITATIVE HISTOCHEMICAL INVESTIGATIONS

W. SANDRITTER and M. SCHREIBER - Frankfurter Zeitschrift für Pathologie 68:693, 1958

A qualitative histochemical study of sputum cells was done in an effort to overcome the well-known difficulties of cytologic diagnosis of lung cancer. Twenty-one methods were employed. Among the stains for nucleic acids, gallocyaninchromalum proved quite satisfactory in bringing out the increased cyanophilia of tumor cell nuclei.

Experiments to stain tumor cells electively, by digesting of nucleic acids in normal cells, were not successful. Reactions for proteins, carbohydrates and fats, such as the tetrazonium reaction, PAS or Sudan were not helpful in sputum diagnosis.

Methods for enzyme determination such as phosphamidase, acid phosphatase and nucleotidase stained tumor cells quite well, but failed to sufficiently set them apart from normal cells. Enzyme reactions are not reliable enough to provide diagnostic advantages. (Authors' abstract.)

HISTOCHEMISTRY OF CELLS IN THE SPUTUM. II. QUANTITATIVE ULTRAVIOLET MICROSPECTROPHOTOMETRIC DETERMINATIONS OF NUCLEIC ACIDS

W. SANDRITTER, D. MÜLLER, H. SCHÄFER and H. G. SCHIEMER - Frankfurter Zeitschrift für Pathologie 68:710, 1958

Quantitative ultraviolet microspectrophotometric determinations of the nucleic acid content of normal and pathological cells were done to find out whether or not photometric methods offer a possibility for improving cytological diagnosis.

From our own data and from those published by others we found, that in normal tissues or smears there are maximally 12.5% cells whose nuclei show a nucleic acid content higher than normal. In most tumors, on the other hand, the number of nuclei whose nucleic acid content is elevated, is close to 10%. Only two out of 50 tumor cases showed a behaviour resembling normal tissue. The distribution of nucleic acid in

normal and malignant cells follows certain rules which are based on the number of chromosomes (stem line principle). Further investigations will be necessary to evaluate the possibilities of photometric cytodiagnosis. (Authors' abstract.)

HISTOCHEMISTRY OF CELLS IN THE SPUTUM S. III. DRY WEIGHT DETERMINATIONS BY INTERFERENCE MICROSCOPE

W. SANDRITTER, H. G. SCHIEMER, W. ALT., D. MÜLLER and E. BEHROUZI - Frankfurter Zeitschrift für Pathologie 69:167, 1958

Dry weight determination of normal and malignant cells of the bronchial system were done, using a Baker type interference microscope.

RESULTS

1. The dry weight of tumor cell nuclei is higher than in average normal cells. In four out of ten cancer cases the dry weight was not elevated. The dry weight readings for different normal and malignant cell types are arranged around average values. The number of cells studied was not sufficient to verify the presence of dry weight duplication series, our data suggest this possibility.

2. The dry weight concentrations of normal and tumor cells are almost equal. Therefore, the higher dry weight of tumor cells must be due to increase in volume which in turn would mean, that they contain more water (absolute values) than normal cells. This is also true for the cytoplasm.

3. Nucleoli exhibit the highest dry weight percentagewise (relation nucleus:cytoplasm:nucleolus = 1:0,2-0,5 : 2,8-12,6).

4. The relation of total nucleic acids to dry weight in normal nuclei ranges from 1:3 to 1:6.

5. The relations between area and volume of nuclei and cytoplasm of normal and malignant cells follows the pattern of an exponential function. The mathematical aspects of this function can be dealt with by use of the allometric growth-formula. Single cells then behave negatively allometric, i.e., as the volume rises the water content increases faster than the dry weight. This trend is more strongly exhibited by cancer cells than by normal ones.

6. In normal cells there is no correlation between nucleic acid content and volume of cells. In these cells the volume depends on the dry weight. In cancer cells we found a positive correlation (exponential function) between volume and nucleic acid content.

7. The interference microscope offers advantages for diagnostic cytology. The cell pictures are rich in detail and contract, especially when polychromatic light is being used. In addition, there is the possibility of determining the dry weight by simple methods. (Authors' abstract.)

TMK-101 (TURK) : A NEW MULTICOLOR STAINING TECHNIQUE FOR OFFICE PRACTICE

NURI SAĞIROĞLU - Am. J. Obst. & Gyn. Date of acceptance for publication, February, 1959

Using Sheaffer Pen Company's inks, a mixture of dye is prepared to stain cervical smears for diagnostic and research purposes.

For the preparation of TMK-101 (4% solution), add 5 cc permanent Blue-Black No. 232 ink and 0.5 cc permanent Red No. 032 ink to 250 cc distilled water in a bottle; shake gently; pour desired quantity into a staining jar.

Material to be examined is obtained with the Ayre wooden spatula and spread thinly on a slide. Without fixation, the slide is immediately placed in the staining solution; 15 minutes is a minimal time to complete staining. The slide is then removed and mounted, guarding against the formation of air bubbles. The same dye solution is used as mounting medium.

TMK-101 dye solution has great staining capacity and is extremely valuable in cytologic studies. It has been used more than 5000 times successively, by the author, on material obtained from 315 patients. Diagnostic value equals other stains currently used. No cellular shrinkage nor loss of detail results when smears are stained in TMK-101. Structure of cells and microorganisms acquire brilliant multicoloration.

Simple, economical preparation and utilization techniques make TMK-101 useful for cervical cytology practice and research application. (Author's abstract.)

OTHER PHASES OF CYTOLOGY

CYTOLOGICAL DETERMINATION OF THE SEX CHROMATIN FOR THE PRE-NATAL SEX DIAGNOSIS (DETERMINAZIONE CITOLOGICA DELLA CROMATINA SESSUALE PER LA DIAGNOSI PRE-NATALE DEL SESSO)

G. V. VALENTI and U. CITTI - Monitore Ostetrico-Ginecologico 28: , 1957

After a review of the principles of the method, its technical data and its importance in the different fields of medicine, the authors relate the results obtained in the cytologic determination of sex in the prenatal period, from the amniotic fluid. The results are sufficiently exact and quite interesting, although the method is not easily applicable. (Authors' abstract.)

A RAPID CYTOLOGICAL METHOD FOR THE DIAGNOSIS OF MEASLES

A. J. BEALE and W. CAMPBELL, J. of Clin. Path. 12:336, 1959

The method of exfoliative cytology has been applied to the diagnosis of measles. In measles, giant cells are present in the respiratory epithelium in the catarrhal stage. These cells are present in sputum which can be collected quite easily from adults. In children sputum is best obtained by suction from a polythene tube inserted along the nose into the posterior nasopharynx. The sputum is spread on a slide which is either fixed in ether-alcohol mixture and stained by Papanicolaou's method or air dried and stained with methylene blue. Either method is satisfactory but the best preparations are obtained by Papanicolaou's technique using a fixed smear. In measles, large syncytial masses are seen. The giant cells most probably arise from fusion of the cells of the respiratory epithelium. A small trial of the method was conducted from which it was concluded that no false positives occur but that the sensitivity of the method is rather low. It was thought that this method enables a diagnosis of measles to be made during the catarrhal stage of the disease, up to 5 days before the appearance of a rash. The method may be of value in the diagnosis of measles in children's institutions. (Authors' abstract.)

WANTED OR AVAILABLE

It is the purpose of this column to promote international exchange of cytologists and cytotechnicians, to inform them of open permanent positions, and to inform employers of available cytology personnel. Persons interested in obtaining permanent positions as cytologists or cytotechnicians or in obtaining temporary fellowships in cytology (teaching, exchange, or training fellowships), and individuals or institutions offering such positions or openings are invited to write giving full information to: ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A. Information supplied will be held strictly confidential.

While information received is subject to editing so that it conforms to the style of ACTA CYTOLOGICA, ACTA CYTOLOGICA cannot and do not assume responsibility for statements made by contributors.

OFFRES ET DEMANDES

Cette rubrique est destinée à favoriser l'échange international de cytologistes et de techniciens en cytologie. Elle renseignera sur les places permanentes vacantes et informera également sur le personnel cytologique disponible. Les personnes désirant obtenir une place permanente de cytologiste ou cytotechnicien, ou faire un stage temporaire en cytologie (enseignement, échange, training), et les instituts ou personnes offrant de telles places sont invités à écrire aux ACTA CYTOLOGICA (5841, Maryland Avenue, Chicago 37, Illinois, U.S.A.) en donnant tous les détails. Les informations reçues auront un caractère strictement confidentiel.

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STELLENANGEBOTE UND STELLENGESUCHE

Mit dieser Rubrik soll internationaler Stellenaustausch und Stellenvermittlung für Zytologen und zytologisch-technische Assistenten angebahnt werden, indem über offene Stellungen und über verfügbares Personal berichtet wird. Zytologen und zytologisch-technische Assistenten und Assistentinnen, die an vorübergehenden (Lehrstellen, Austauschstellen, Lernstellen) oder dauernden Anstellungen interessiert sind, und Personen oder Institutionen, die derartige Stellunge zu vergeben haben, sind gebeten an ACTA CYTOLOGICA (5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A.) zu schreiben und möglichst genaue Einzelheiten anzugeben. Die erhaltenen Auskünfte und Einzelheiten werden streng vertraulich behandelt.

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El propósito de esta sección es promover el intercambio internacional de citólogos y técnicos en Citología, informar de vacantes en puestos permanentes, y de personal citológico disponible. Las personas que estén interesadas en obtener becas temporales, o puestos permanentes como citólogos o técnicos en Citología (Enseñanza, Intercambio, Becas de aprendizaje), y, asimismo, las personas o instituciones que puedan ofrecer tales puestos, deben escribir a ACTA CYTOLOGICA (5841 Maryland Avenue, Chicago 37, Illinois, USA) aportando información completa. Esta información será estrictamente confidencial.

Cuando las informaciones recibidas sean para su publicación en ACTA CYTOLOGICA, la Revista, no puede asumir, ni asume, la responsabilidad de los informes o afirmaciones hechas por los contribuyentes.

CYTOLOGISTS AND CYTOTECHNICIANS WANTED

CYTOLOGIST IN CHARGE WANTED for University Department of Obstetrics and Gynecology. Candidates must be Doctor of Medicine interested in teaching, research and special studies in Exfoliative Cytology. Foreign candidates must have mastered the English language and be acceptable to the local Medical State Board. The appointment would be initiated with the rank of of instructor with an annual salary of $7200.00, subject to merit increases in salary and promotion in rank. Candidates are requested to submit their curriculum vitae with bibliography (including available reprints of publications) and three references (out of whom at least one must be from the United States) to Russel R. de Alvarez, M. D., Professor and Executive Officer, Department of Obstetrics and Gynecology, University of Washington, Seattle 5, Washington, U.S.A.

MEDICAL RESEARCH TECHNOLOGIST WANTED trained or willing to be trained as a cytotechnician for work in the Department of Obstetrics and Gynecology at L.S.U. School of Medicine; Louisiana State Civil Service Employee; starting salary $3500.

QUALIFIED CYTOTECHNICIAN WANTED full-time for Department of Pathology of Medical College of Georgia, Augusta. Opportunities for research and teaching in medical school environment. New laboratories, well-equipped and air-conditioned. Attractive salary.

Apply: Dr. L. D. Stoddard, Professor of Pathology, Medical College of Georgia, Augusta, Georgia, U.S.A.

RESEARCH FELLOW IN GYNECOLOGICAL PATHOLOGY WANTED. The Baptist Memorial Hospital, Jacksonville, Florida, will offer a Research Fellowship in the general area of research in gynecologic pathology to begin in the summer of 1959. The hospital currently operates 272 beds. It was opened in September, 1955, in a new air-conditioned building directly on the St. Johns River. The laboratories are attractively located on the second floor with separate laboratories for Histology, Bacteriology, Hematology, Clinical Microscopy and Chemistry. A new research laboratory with animal facilities is located on the sixth floor. The hospital is approved for residency training in Pathologic Anatomy and Clinical Pathology. Requirements: Graduation from an approved medical school, one year of approved internship, and at least one year of approved residency training in pathology or in obstetrics-gynecology. Stipend: A stipend of $3800 per year with an additional $350 per year for one to two dependents. No maintenance is furnished. Uniforms are not furnished but are laundered without charge.

Applications and inquiries should be addressed to: Alvan G. Foraker, M.D., Pathologist, Baptist Memorial Hospital, 800 Miami Road, Jacksonville 7, Florida, U.S.A.

RESIDENCY FOR FOREIGN GRADUATE (Other than French) in Obstetrics and Gynecology offered in the Maternite de l'Hopital de Creteil, Paris, France. The applicant should have experience in surgical obstetrics and gynecology (caesarean sections, forceps, hysterectomy) prior to application. The applicant will receive training in general obstetrics and gynecology and will have the opportunity of working in the laboratories, especially in the cytology laboratory of Professor Jean de Brux. The opening for this position will be either November, 1959, or April, 1960, and is for the period of one year. The monthly salary is 45.000 to 50.000 French francs. For further details write to Docteur A. J. Bret, Chef de Service de la Maternite de l'Hopital de Creteil, 53 Avenue de Saxe, Paris 7, France.

CYTOTECHNOLOGIST OR REGISTERED MEDICAL TECHNOLOGIST WANTED. 250-bed general hospital in a college community of approximately 4,000 people wants cytotechnologist or registered medical technologist for its laboratory. 40-hour working week with occasional weekend duty. Residence for technicians available. Two weeks annual vacation, plus two weeks sick leave. Salary for this position is open. Any interested persons should write to: Dr. Elizabeth French. Director, Clinical Diagnostic Laboratories, Mary Hitchcock Memorial Hospital, 2 Maynard Street, Hanover, New Hampshire, USA.

CYTOLOGIST (EXFOLIATIVE) NEEDED as chief screener and supervisor of growing cytology laboratory. Approved ASCP school of cytotechnology. New modern laboratory in one of the largest private hospitals and research centers in the Midwest. Outstanding employee benefits. Salary $5000-6000, depending upon qualifications. Write to: Dr. Otto Saphir, Pathology Department, Michael Reese Hospital, Chicago 16, Illinois, USA.

CYTOLOGISTS AND CYTOTECHNICIANS AVAILABLE

CYTOLOGICALLY TRAINED RESIDENT, Male, citizen of West Germany.

Cytology Training:	Trained for one year in a teaching laboratory in the United States (approved by the American Board of Pathology for training Pathologists) and has finished a complete internship in the United States.
Wanted:	Appointment in a University Hospital in Germany or in the United States of America. Available as of January 1, 1960.
Code No.:	MD-1-1959, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

CYTOTECHNICIAN, Female, Age: 32, citizen of West Germany, single, registered medical technician.

Cytology Experience:	Chief-Cytotechnician 7 years in cytology laboratory of a University Department of Obstetrics and Gynecology in Germany. Experience in cancer cytology, endocrinological cytology and hematology.
Wanted:	Exchange fellowship for a period of several months with a cytology center in the United States of America, Brazil or Argentina. Will return to present position upon completion of fellowship.
Code No.:	RU 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

MEDICAL TECHNOLOGIST, Female, citizen of the United States, single.

Cytology Training:	Extensive training in anatomy and histology of female genital tract, hormonal evaluations, atypical cytology and carcinomas of the female genital tract with additional practical experience in the handling and processing of cytological material.
Wanted:	European position as a gynecological cytotechnologist, beginning July 1.
Code No.:	GAG 3/1/59, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, USA.

TRAINED CYTOTECHNOLOGIST AVAILABLE FOR SCREENING. Cytotechnologist with ten years of experience wants screening at home for pathologists and physicians. Will accept processed slides only. Charge per case. Code no.: MIDDLEWEST 3/1/59, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, USA.

BUSINESS MATTERS
OF THE INTERNATIONAL ACADEMY

FROM THE OFFICE OF THE PRESIDENT

In accordance with the Bylaws of the International Academy Article I, Section 3, an

INTERNATIONAL CONGRESS OF EXFOLIATIVE CYTOLOGY

is scheduled for August 31 to September 2, 1961, immediately preceding the Third World Gynecology Congress. The place of the meeting will be Vienna, Austria.

Extracts from the Bylaws concerning the Scientific Session (Article 1, Section 10) : ". . . Papers presented at the Scientific Session shall be original papers which have never been presented or published. Material which has already been published or presented elsewhere may be considered in panel discussions. Fifty per cent of the Scientific Session shall be devoted to original papers, and fifty per cent of the time to panel discussions. . . ."

The attendance and participation is not restricted to members of the International Academy.

The Congress of Exfoliative Cytology will deal with, among other general topics, the following two main subjects:

(1) Progression and regression of epithelial abnormalities, and

(2) The hormonal activity as reflected in the vaginal smears in cases of gynecological tumors, breast tumors, and ovarian dystrophy.

In addition to other panel discussions, there will be an extensive discussion on cytological and histological terminologies and definitions.

Application forms for participants may be obtained from

The Offiice of the Secretary
International Congress of Exfoliative Cytology
666 Elm Street
Buffalo 3, New York, U.S.A.